APPENDIX C

PREMEETING COMMENTS PREPARED BY EXPERTS

Shrimp Virus Peer Review Workshop

Premeeting Comments

Arlington, VA
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Charge to Experts

Please prepare your written comments to address the questions posed below. The first 18 questions are organized based on elements of the ecological risk assessment process, as described in the shrimp virus report (which is located in the Minutes of the Stakeholder Meetings on the Report of the JSA Shrimp Virus Work Group). Questions 19 to 22 ask your opinion about the need for a comprehensive risk assessment; this topic will be discussed during the last half-day session at the workshop. You may also address other issues that you feel are important. All written premeeting comments will be distributed to other experts prior to the workshop and may be included as an appendix to the final workshop report.

Management goals, assessment endpoints, and the conceptual model

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquiculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

Viral stressors and factors regulating shrimp populations

This topic includes basic information about shrimp viruses as well as the full range of natural and anthropogenic factors that regulate shrimp populations. Questions for consideration:

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquiculture operations?

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

Viral pathways and sources

The shrimp virus work group considered aquiculture and shrimp processing to be the primary pathways of concern leading to exposure to pathogenic shrimp viruses, but it also identified a number of other potential pathways. Some related questions are listed below.

Aquiculture

9. U.S. aquiculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquiculture operations as a source for the virus?

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquiculture and wild shrimp populations, with regard to shrimp viruses?

Shrimp processing

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Other potential sources and pathways

13. After considering the sources addressed in the shrimp virus report, what sources other than aquiculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

Stressor effects

These next questions. concern the possible consequences to wild shrimp populations and marine communities from exposure to pathogenic shrimp viruses.

15. How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquiculture into the wild in South America?)

16. There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

18. How important are potential viral effects on non-shrimp species?

Comprehensive risk assessment and research needs

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

20. What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp populations models), and what would be the likely time frame and cost?

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

22. Summarize the critical research needs for completing such a risk assessment.

Peer Reviewer Comments

Ned Alcathie

NED ALCATHIE

12/17/97

Responses to Charge to Panel members (Shrimp Virus Workshop):

Management goals, assessment endpoints, and conceptual model

1. The management goal appears to adequately reflect the shrimp virus problem.

2. Modifying the assessment endpoints to emphasize potential risks to non-shrimp organisms, the estuarine ecological system or the aquiculture industry appears to be very wide in scope. However, information on these areas of concern may be useful during determinations of final endpoints.

3. In order to have for the risk assessment to be manageable I feel it should remain narrowly focused. Seafood processing in coastal areas should be considered since this may be possibly a significant source of introduction of viruses into the wild shrimp population.

Viral stressors and factors regulating shrimp populations

4, 5, 6, 7, 8- unable to answer with any degree of certainty, best left to those with backgrounds in virology.

Viral pathways and sources

Aquiculture

9, 10- unable to answer

NED ALCATHIE

Shrimp processing

11. As far as I know little if any information exists which would support or refute the importance of shrimp processing as a potential source of the virus. It is possible that the processing industry is contributing to the introduction of viruses since many facilities in coastal areas discharge untreated water used in processing directly into rivers, bays and the Gulf of Mexico.

12. It is doubtful that retailers would constitute more than a minimal risk.

Other potential sources and pathways

13. Unable to answer

14. The only processor of shrimp plant wastes (shells, heads, etc.) that I am familiar with uses a drying process that begins at approximately 1000 deg F, 20-30 minutes later the end product exits the dryer at approximately 200 deg F, with a moisture content of 8-9%. This would seem to rule out a potential source of the virus.

Stressor effects

15, 16, 17, 18- unable to answer

Comprehensive risk assessment and research needs

19, 20, 21, 22- unable to answer

Acacia Alvicar-Warren

Management goals, assessment endpoints, and the conceptual model

1. How well does the management goal reflect the dimensions of the shrimp . virus problem?

The shrimp virus problem is a very broad problem with many dimensions both in and outside the shrimp industry. As long as the stated management goal of "Prevent the establishment of new disease-causing viruses in wild populations of shrimp" is interpreted very broadly, I agree with most of it with two exceptions. First, the geograpfic coverage should be enlarged to include the US Pacific coast. Second, because contibuting factors to the shrimp virus problem may reside in many industries and activities seemingly unrelated. to the shrimp industry, the portion of the management goal that refers to "minimizing possible impacts should not be limited to "shrimp importation, processing, and aquiculture operations". but instead should be broadened to include minimizing the impacts on all industries and activities that are found to contribute to the shrimp virus problem. For example, the destruction of estuarine habits and environmental degradation might prove to be a significant source of new viruses.

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrirnp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquaculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

The two assessment endpoints suggested by the Shrimp Virus Work Group should be the focal points for the ecological risk assessment

1. "Survival, growth, and reproduction of wild penaeid shrimp populations", and

2. "Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations"

Point 1, however, should be broadened to include the US Pacific coast.

Point 2, I would agree that there is a need to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system. However, a comprehensive epidemiological / genetic study should first be performed in order to obtain baseline information on both the genetic structure and the prevalence of the viruses in the natural penaeid shrimp populations.

A healthy estuarine ecological system will supply the virus-free wild shrimp stocks needed to support the aquiculture industry.

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as altemative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

I agree with this statement and recommend that it should be broadened to include all stressors associated with "alternative land uses and seafood production methods in coastal areas" including (1) habitat destruction, (2) chemicals and environmental contaminants, and (3) introduction of exotic species and release of cultured stocks.

For point (l), the impact of habitat (mangrove) destruction on the production - rate of wild shrimp is well documented (Jothy, 1984, Lahman *et al*, 1987; Paw and Chua, 1991). The presence of mangroves has been found positively correlated with nearshore yield of shrimp (Paw and Chua, 1991). The loss of mangroves translates into a direct loss of habitat and species diversity of an unknown magnitude and has been suggested as the dominant cause of the decline in the abundance of wild shrimp postlarvae in Ecuadorian estuaries (Lahman *et* al., 1987; Twilley, 1989; Parks and Bonifaz, 1994).

For point (2), intensive levels of industrial shrimp farming has also brought about an increased use of chemicals and other products which can cause marine pollution (Primavera, 1993). Mortalities and morphological deformities in shrimp larvae caused by the widespread use of such chemicals as oxytetracycline, nitrofurans, chloramphenicol, malachite green and copper sulfate have been reported (ibid.).
Pathogenic bacteria causing luminous vibriosis in shrimp larvae were found to be resistant to antibiotics and it is now a serious problem in various countries in Southeast Asia. The direct effects of these chemotherapeutants and antibiotics on humans constitute a public health concern.

For point (3), exotic shrimp species have been introduced to various countries for many decades with ecosystem-wide repercussions. The problems include hybridization, competition, introduction of new diseases, or lead to genetic changes in the wild population (Rosenthal, 1980; Brock, 1992; Sinderman, 1992). The release of exotic shrimp from cultured populations has been documented in the Atlantic coast of the United States (Wenner and Knott, 1992) where native Pacific stocks of *P. vannamei* and presumably escapees from a Shrimp farm, were found in offshore samples. The P. *vannamei* in the Atlantic coast was estimated to be at -7% of the total shrimp sampled. The presence of a sexually mature *P. vannamei males* off South Carolina suggested the potential for interbreeding (Wenner and Knott, 1992). Moreover, considering that some cultured stocks are potentially inbred and genetically susceptible to viral diseases (Alcivar-Warren *et*

al, 1997) there is a possibdity that they could also serve as a reservoir for rapid multiplication of the viruses and spread of diseases.

Viral stressors and factors regulating shrimp populations

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquiculture operations?

Is the only way to measure viral threats to date. Research is needed to demonstrate virus infectivity in samples from aquaculture and wild shrimp populations.

5. How **likely** is it that exposure of wild shrimp populations **to** viral diseases could **lead** to the development of immunity and reduced effects on populations survival over time?

Basic research on the immune system of shrimp needs to be performed before this question can be addressed.

Research funds should be directed to study both immunology and genomics of shrimp. Studies on the molecular biology and evolution of shrimp viruses as well as the cellular mechanisms involved in the recognition and interaction of the virus with the host genome will help to understand species-specific disease expression.

It is possible that because of the apparent lack in shrimp of the major immune (T and B) cells present in fish and other vertebrate species, a mechanism of "adaptive immunity" has evolved in shrimp species which may reduce the effects of viruses on population survival over time. This hypothesis need to be tested first.

6. How can the strong influence of both natural and non-viral **anthropogenic** factors on shrimp populations be separated from risks associated with viral **stressors?**

I doubt that the influence of both natural and non-viral anthropogenic factors on shrimp populations can be separated from risks associated with viral stressors.

The possibility exists that the environmental pollutants (e.g. heavy metals and pesticides) present in the estuarine ecosystem are of such magnitude that they also weaken the shrimp immune system making the animals even more susceptible to a viral pathogenic attack. Pollutants like the heavy metals mercury and cadmium are also known to accumulate in marine organisms, including shrimp, and cause rapid genetic changes (Nevo *et al.*, 1986). Moreover, the impact to the natural populations caused by the release of cultured stocks also

need to be considered in the risk assessment. Some cultured stocks are potentially inbred and genetically susceptible to viral diseases (Alcivar-Warren *et al.*, 1997) and may serve as a reservoir for rapid multiplication of the virus and disease transmission.

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Nothing should be ruled out pertaining to virus diseases. More basic research is needed in order to understand the biology and mutation rate of the viruses.

Viral samples should be stored to maintain a shrimp virus database for future studies of infectivity, mutation rates and potential transmission to other species.

Government agencies should begin monitoring / inspecting shrimp imported for human consumption. Other shrimp diseases (vibrios in particular) should not be ignored as they represent a real threat to human health.

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

More research is needed to develop sensitive molecular (quantitative RT-PCR) and immunological (antibodies) techniques to screen for the viruses (particularly TSV and YHV) in various samples including tissues from wild populations, manufactured feed and environmental media. This is an important issue for the risk assessment as viral detection can be tissue-specific and various tissues may need to be tested from each animal. For example, sensitivity of detection of WSSV by PCR depends *on the* tissue selected, being more sensitive in hepatopancreas and pleopods than in hemolymph of *P. monodon* DNA (Lo, personal communication).

Viral pathways and sources

9. US aquiculture operations have had problems with viral diseases for several years, How does information from local wild shrimp populations support or refute the importance of aquaculture operations as a source for the virus?

Though it appears that the guidelines recommended by the US Marine Shrimp Farming Program have not always been followed by the aquiculture industry, there is no published data to support or refute the importance of aquiculture operations as a source for the virus, nor do I believe that we would be able to document it with the current detection technologies and lack of information about the prevalence of the viruses in the wild populations.

I recommend that the analysis of the natural population be performed first. The first step should be to develop epidemiological and genetic baseline information. See my comments under questions 4, 15,20 and 22.

Also, we need to study the possibility that cultured stocks, if released into the estuarine environment, may transfer these and other unidentified viral pathogens and may influence the fitness of the natural shrimp populations. See my comments under question 10 below.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquiculture and wild shrimp populations, with regard to shrimp viruses?

This is perhaps one of the most important questions that remain to be answered. If it proves to be the case the wild populations are immune or much less susceptible or able to recover on their own from viral attacks, then that would seem to argue strongly that either shrimp farming procedures or shrimp broodstock breeding programs need to be changed,

No scientific research has been performed to date to document the impact of domesticated populations into the natural populations. It is possible that viral diseases may spread if cultured stocks are accidentally or intentionally released into the wild. Even if these cultured stocks are free of the virus, their susceptibility y could make them a reservoir for the virus to mutipl y even faster

International efforts should be made to help other countries to properly discard diseased shrimp from viral epidemics, effluent from aquaculture facilities, waste from processing plants and untreated human sewage from local communities surrounding the estuary ecosystem.

Shrimp processing

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

Unable to make an statement at this time. We need a baseline epidemiologicai / genetic study on he natural population first.

Research funds are needed to examine the impact of releasing cultured stocks (potentially inbred and genetically susceptible to viral diseases) into the natural population, The American industry should also take precautions when exporting these stocks to other countries in Latin America or across continents.

A genetic risk assessment from the other country should be required first. This movement of shrimp needs to be more closely monitored.

If we are really serious about preventing viral and other diseases in the US wild shrimp populations, and protect human health, we should begin immediately a federal monitoring progam aimed at screening for the presence of the viruses in the shrimp food and in live and frozen shrimp brought into the US.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Yes - at least with a small study that would make a reliable estimate as to whether they are a large or small contributing factor. If large, continue study. If small, stop.

May need intemational cooperative agreements with exporting countries.

Other potential sources and pathways

13. After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

Other sources most critical for evaluation in a risk assessment of shrimp viruses are:

- habitat destruction and environmental contaminants
- impact of potentially inbred and genetically susceptible cultured stocks on the wild populations
- international trade in brood and seed stocks
- maufactured feed - fish meal from South American countries, is used to prepare shrimp feeds in Southeast Asia -is the food processed at >IOO°C?
- what about other species (including human) as sources?
- for a pathway, what about other vehicles such as human sewage or the wastes of other industries from countries surrounding US coastal waters?

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

Don't know. Additional research is needed to demonstrate that manufactured shrimp feed is a potential virus source and should be further investigated, particularly considering the preliminary information about the possibility that infectivity of TSV is maintained after boiling at 100°C (Lotz, USMSFP Progress Report, preliminary information), This is important because a large percentage

of the supply of fish meal and other ingredients used by the shrimp feed industry originates from South American countries where TSV disease is endemic.

Stressor effects

15. HOW should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? For examples, what was the role of IHHNV in the decline of shrimp populations in he 1980's in the Gulf of California? What about TSV release from aquiculture into the wild in South America?)

There is not nearly enough evidence yet on the effects of introduced viruses on wild shrimp populations for valid conclusions to be drawn.

This is probably the most important area for research and the following should be addressed:

- Publish yearly census of wild shrimp populations.
- Save virus samples year by year in order to determine if the viruses have mutated or if shrimp really have developed immunity - need to develop monoclinal antibodies to help differentiate virus strains.
- Regarding "the role of IHHNV in the decline of shrimp populations in he 1980's in the Gulf of California", how can a population come back from a virus attack such as IHHNV after 7 years? Are there good records of the yearly census of the wild populations in the area? May be other non-virus, environmental, anthropogenic factors (e.g. destruction of the mangrove habitat, weather parameters, salinity, El Nine, etc.) influenced the population decline.
- The statement about "TSV release from aquiculture into the wild in South America" should be considered with caution. While some cultured shrimp stocks are known to have low levels of genetic diversity (Garcia *et al.*, 1994; Sunden and Davis, 1991) and are genetically susceptible to most viruses (Alcivar-Warren *et al.*, 1997) a proper monitoring of the industry trade activities and the epidemiology/ genetic structure of the wild South American shrimp have not been done. The potential impact of environmental degradation on the health of wild shrimp populations on the Gulf of Guayaquil, Ecuador is well documented (see my comments under question #3 above). It is possible that environmental stressors (water quality and toxicants like heavy metals and PCBs) affected the immune system of the wild penaeid populations making them susceptible to Taura Syndrome epidemics and other viral and bacterial diseases.
- The possibility that cultured stocks released into the marine environment may impact the natural population and should be included as an endpoint of the risk assessment.

- The impact of human activities (sewage treatment, etc.) industrial toxicants (oil and agricultural runoffs) in both US and Mexico communities surrounding the Gulf of Mexico and Pacific coastal waters.

16. There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in US waters. How should this data gap be evaluated in a risk assessment?

This is a key gap and should be evaluated first through a comprehensive epidemiological / genetics study that includes the participation of research teams that include epidemiologists, virologist, immunologists, veterinarians, marine biologists and populations geneticists.

The lack of basic data on background levels of pathogenic shrimp viruses and the genetic structure of the shrimp natural populations should be the first issue to be addressed in the risk assessment.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Until we have a baseline information on the genetic structure of the wild shrimp populations and the presence / absence of different viruses, we will not be able to make this interpretation.

18. How important are potential viral effects on non-shrimp species?

Research is needed and baseline information on the presence of the viruses on non-shrimp species should be obtained first.

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

Yes - if it is done broadly enough. If the risk assessment is done in a narrow fashion (i.e., concentrating only on the aquiculture shrimp industry), then it will likely not be very useful.

20. What type of assessment should be conducted next (e.g. quantitative risk estimates using shrimp populations models), and what would be the likely time frame and cost?

A more holistic approach to quantitative risk assessment is needed. At all costs, the risk assessment should be performed immediately and focus first on the development of baseline information on epidemiology and genetic structure of the natural populations. The following goals should be addressed:

1. study the genetic structure and effective population size of the wild penaeid species in US coastal waters *(P. aztecus, P. setiferus* and *P. dnorarum)* as well as the species used by the aquiculture industry *(P. vannamei* and *P. stylirostris).*

2. determine the prevalence of viral (DNA and RNA) sequences in the same samples of wild shrimp from which the genetic data is derived.

3. maintain a genetic database of shrmip viral sequences obtained from different geographic regions representatives of different estuarine habitats.

This will be a long-term and expensive project aimed at documenting population changes in time and space but it should be performed if we are really serious about preventing diseases and protecting the wild shrimp populations. It will be impossible to tell if you are having success in managing disease without the baseline information.

In the meantime, government agencies should join efforts to put a moratorium on the importation of foreign shrimp (for all uses, food and aquiculture) until exporting countries agree that their frozen shrimp products need to be tested (similar to the current practices with cattle diseases).

At all costs, the industry should also be proactive regarding environmental issues and controlling spread of diseases by stopping the movement of shrimp species across regions. For example, *P. stylirostris* has been moved from the Pacific coast to the Atlantic coast of Venezuela. The stocks used by the industry should also be genetically diverse and free of diseases, pond by pond.

With high fecundity species such as shrimp, immunity may be on a population basis rather than an individual basis. If this is the case, then it might mean that we need to fundamentally change the approaches that we use in developing shrimp breeds for use in acquaculture. For example, it might be better to use tagged offspring (using molecular markers) from a large group of genetically different individuals / species in a pond rather than from a few.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

Yes.

22. Summarize the critical research needs for completing such a risk assessment.

The most critical areas of research needed are:

1. Perform an epidemiological / genetics study to develop baseline information on the wild shrimp population. Yearly census of the wild populations is needed.
2. Asses the risk posed to the wild shrimp populations because of accidental or intentional release of cultured stocks. The first step is to know the structure of the wild populations in their natural range.
3. Research the impact of other stressors (e.g. habitat destruction, PCBs and heavy metals, exotic introductions, weather changes, El Nino, gene flow, salinity, processing plants and pond wastes, infected bait shrimp, human waste, non-shrimp hosts/carriers) which may affect the health of natural shrimp populations.
4. Fund studies on shrimp immune-genetics.
5. May need to fund Mexican participation on the first three issues above. A "fortress America" approach will not work.

Finally, consumers should be reassured that the food we are eating is properly inspected. Federal agencies should better define and coordinate their activities on importation, interstate movement, release of live animals and waste management in order to prevent future threats to wild shrimp populations, aquatic ecosystems and aquaculture and to protect human health.

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Mark Berrigan

Comments Shrimp Virus Work Group

Mark Berrigan

1. The draft management goal reflects the ecological and economic elements associated with the potential establishment of marine shrimp viruses. The draft management goal does not include scientific confirmation that a specific problem exists or its specific ecological consequences.

2. The proposed endpoints are very broad. Without previous understanding of the potential problems associated with introducing shrimp viruses, the reader might not make a connection between the potential risks of disease and the proposed endpoints. Should the stressor of concern be incorporated in the asessment endpoint?

3. Initially, the risk assessment should focus on potential ecological implications. Broadening the scope of the risk assessment should be a consequence of preliminary analyses, findings and recommendations.

4. Relevance is an important consideration, and should be determined through good science.

5. Uncertain -

6. Uncertain -

7. Uncertain -

8. Uncertain -

9. There is little scientific information to confirm or refute the occurrance of epizootics among wild shrimp populations associated with naturally occurring or introduced viruses. Obviously, resolving this issue is problematic. However, this is a critical element in determining the direction of the risk assessment. Assessing the likelihood of potential epizootics should be an assessment endpoint, at least in the initial phases of the risk assessment plan.

10. Uncertain -

11. As far as I know, there is no strong link between processed shrimp or process wastes and shrimp epizootics. However, since the imports of shrimp compromised by viral diseases has probably increased, and new viral diseases have been manifested in the last several years, it. may not be appropriate to suggest that "if a problem existed, it would have been identified by now".

Also, it becomes important to determine if it is common practice for growers to harvest shrimp that manifest diseased conditions and export them to particular markets. It is understandable that certain international markets would not accept deseased shrimp when quality and appearance have been compromised. Other markets may not make this distiniction.

12. Quality control and quality assurance will be problematic in the import, proccessing, distribution and marketing sectors. Retailers probably make the assumption that the product is safe if nothing of public health significance is associated with the product.

13l Although other pathways may be plausible, focusing on alternative sources will detract from the critical issues- "does importing shrimp (dead or alive) pose a threat to natural populations and aqaculture?" While these other pathways are realistic - management will be problematic.

14. Supplemental feeds that incorporate processing by-products, such as solar-dried shrimp exoskeletons may be a potential pathway. Certain extruded rations may, be produced at low temperatures and pressures that might not destroy pathogenic viruses.

15. Anecdotal information should be considered carefully, and should not be treated as fact. However, this type of information may be useful in identifying underlying problems. For example, reports from fishermen may be helpful in identifying fishery trends. Likewise, scientific information should be carefully scrutinized and interpreted before specific results are applied to different scenerios.

16. The lack of scientific data associating viral diseases with epizootics among natural shrimp population is a major shortcoming in establishing what risks actually exist: for example, identifying threatened populations, determining exposure levels, and characterizing ecological consequenses remains a problem.

17.

18.

19. Aquaculture: A comprehensive risk assessment will assist resource managers in developing best managment practices for marine shrimp aquaculture operations. Types of aquacultural pracitces, site selection, and regulations could be developed based on known risks. For example, low-risk aquacultural activities in teh high-risk scenerio would not be suitable in all circumstances, while implementing specific management decisions for activities that fall between the low and high risk scenerios should be based on best available information.

20. An assessment of natural shrimp in areas where aquacultural activities are concentrated would be useful, but perhaps difficult. For example, models of shrimp populations on the coasts of Ecuador, Honduras, and Panama might shed some light on the threat to these populations from concentrated shrimp aquaculture. I understand that natural populations in these countries are thriving, and it would be interesting to determine: 1) if taura virus is present in the wild populations, and 2) if affected populations manifest any level of resistance to taura virus. Obviously, there is scientific and anecdotal information that may be relevant, but is not currently being used in a risk assessment format.

21. I think that risk reduction through a range of options is the logical outcome of a risk assessment when risk can be identified and Characterized.

22.

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1. How well does the management goal reflect the dimensions of the shrimp virus problem? While the goal specifically addresses the prevention of new disease-causing viruses, it does not suggest a goal of further understanding and prevention of recurrent virus epizootic events. Further it was suggested in the report to include considerations of alternative marine hosts such as crab, crayfish etc., but no suggestion has been made to address related arthropod viruses such as those infecting insect populations which could readily enter the food chain of shrimp.

2. The tiered method of risk assessment is applicable here as described in the review mareria!s. The focus should remain on preserving the wild populations of shrimp. The corollary to include non-shrimp host susceptibility should remain a concern, but should be placed in the. second tier of concerns along with the aquaculture industry and estuarine ecological impacts.

3. Consideration of any and all pathways that may impact the spread and resilience of viruses should be accepted and given adequate attention. Though the ultimate decisions will be based on what is practical, all potential areas that may in initiate an epizootic event need to be identified and considered for future assessment if necessary.

4. Results on virus infectivity gained from laboratory or aquiculture facilities are valuable, but need to be tempered by the artificial conditions and or animal densities that are typically maintained. The spread of virus in these populations proposes to be far more rapid than would occur naturally in wild populations. Further, in a natural enviroment, a continual dilution of virus due to wave action and tidal exchange would reduce the potential for a localized concentration of virus to occur. An underestimation of virus concentration may also occur in experimental infections due to the protection offered viruses by sediments and or secondary hosts. The relevant value of experimental infections is that it targets specific tissues of infection and the nature of the lesions produced, which may assists in initial diagnosis in wild populations.

5. Given the high mortalities associated with the four viruses specifically addressed in the JSA report, it would seem highly unlikely that an intentional infection of wild populations would result in an overall immunity conferred upon survivors. Moreover, it would seen likely that survivors would be carriers and represent potential vectors that may introduce a virus to a different population leading to sustained infections across many populations as they interact. The only evidence (though not specifically identified as such) of this technique is an extrapolation of the events that transpired from 1990-1994 California with *P. stylirosrus.*

6. The non-viral and natural factors affecting shrimp can be separated from viral stressors only after there has been substantial monitoting and evaluation of these non-viral factors, Much remains unknown as to the impact of effectors such as salinity, temperature etc. in regard

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immigration during postlarval stages of development. A more comprehensive model based on additional research efforts at this basal level are required prior to fully understanding the causal relationships between specific viruses and shrimp. Until such a time, the best we can do is to state as many relationships between viral and non-viral associated risks and be aware of the potential consequences of these interactions.

7. Most human health effects can be removed, except for very isolated incidents. The treatment of waste in a municipal waster treatment system is generally more that adequate to dispose of any threat of shrimp viruses. Most wastewater treatment plants implement several stages to handle such threats of reintroduction of human pathogens back into the population. Steps in processing and disposal have been implemented in most communities to include a series of effluent treatments such as ozonation and aeration prior to discharge into receiving streams, Solids disposal has been addressed in these facilities as well in that many facilities incinerate the solid waste materials. If any threat remains it is in locations where solid waste is landfilled and water run off could reenter a water system co-occupied by shrimp.

8. No. Additional research is required to develop reliable diagnostic detection of viruses in shrimp stocks and further techniques need to be developed for the testing of pond effluents or other waters suspected of containing shrimp viruses. The ideal system of diagnosis would be a cel1 culture system that would allow the testing of a range of sources from a single suspected animal to the determination of virus presence in specific pond. To date the efforts to establish a cell line for diagnostic viral detection *have been* hampered by limited availability of significant quantities of tissues From specific-pathogen-free animals. Other resources that would permit a consistent concentrated effort have also been lacking.

9 & 10. As of this report, no direct causal relationship has been established between outbreaks of virus in aquaculture facilities and the transmission of the virus to a wild population. Though there has not been a direct link established it does not rule out that it has occurred in the pastor will occur in the future. As was mentioned in the JSA report, wild populations have not been adequately monitored. As the capabilities become available to accurately monitor wild populations, and detect viruses in aquaculture discharge, we may be able to track the movement of potential virus infection. This goal would best be achieved in trials employing biomarkers.

11. This is an ever-increasing potential problem especially in light of the fact that Asian markets are exporting ponds after initial traces of virus infection. The disposal of wash waters form port-side processing facilities directly into receiving waters that support any phase of shrimp

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development should be of great concern. Additional solid waste provides 3 protective cover for virus propagation and entry into the shrimp food chain.

12. No. Aside from increasing demand for ultraviolet (UV) treatment of potential infectious agent in other markets, no additional evaluation is warranted. It should be noted that the additional push for UV treatment could also diminish the negative public opinion.

13. The two potential sources or pathways most critical after processing and aquiculture are that of ballast water discharge and secondary or alternative hosts which can harbor the shrimp viruses. The potential impact of the former may be diminished or eliminated if ballast discharges were properly filtered and the filter dried and incinerated.

14. The temperature obtained during feed production, is adequate to eliminate it as a source of virus. However, is a cell culture system were available, soluble extracts could be prepared and checked for active virus.

15. Evidence concerning the introduction of viruses into wild shrimp populations should be interpreted with caution and reserve. No definitive association has been made between the incidents.

16. Though no data is available for background leveis of virus in thc wild populations of shrimp, it would be better to error on the side of caution. in a risk assessment, it would be better to presume that a wider variety and higher numbers of viruses exist in the wild populations. The reason that the viruses have not resulted in epizootic events is due to proper timing of environmental and physical conditions. Furthermore, the lack of adequate monitoring of wild populations may have precluded us from characterizing these events.

17. Monitoring of changes in the wild shrimp populations can be used to interpret the impact of introduced populations by characterizing the latcnt period of the virus in a population after infection in their natural environment. Additionally, if there is an advantage to propagating animals that survive an infection due to some immune advantage, this would allow the true determination of this phenomenon. The immune resistance conferred by an initial infection of this manner at low MOI (multiplicity of infection) could potentially be explained as the population recovery curve were established and animals were randomly screened for viruses. What this scenario does not consider is a multiple pathogen infection.

18. Shrimp virus effects on non-shrimp species is of significant concern as a protected latent storage potential, as well as the possibility of infecting a non-shrimp species through mutation. The ability of a non-shrimp species to harbor a shrimp virus that may re-infect on a recurring basis either seasonally or any time in which the two species interact.

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19. A comprehensive risk assessment will outline the primary, secondary and tertiary factors affecting both wild and cultured shrimp. This format will also point out the research data lacking and potentially bring to light the avenues of research that need to be developed. The assessment will bridge gaps in communication between interested parties to the shrimp economy and will hopefully result in the cooperative exchange of ideas and information toward a common goal.

20. Data gaps are to staggering to proceed directly to a quantitative risk assessment. Which research redirection and monitoring of wild populations for a rninimum of two complete developmental cycles for shrimp, population models could then begin to be developed. The cost of such an endeavor would likely cost several hundred thousand dollars, but would add immensely to the scientific integrity of the model development.

21. As technology is developed to reduce the risk of virus introduction from processing and aquaculture wastewater disposal, a future risk assessment should reward these efforts by factoring in a risk reduction element to the formula similar to the risk assessment model, figure 2 from the Report to the Aquatic Nuisance Species Task Force.

22. In reviewing the materials presented, four key points in research come to mind. First, there is a great need to implement a monitoring program of wild shrimp populations for virus presence and genetic diversity. Second, determination of specific non-shrimp harboring species needs to be looked at. This should include not only other marine species such as the aforementioned crab and crayfish, but also in non-marine arthropods. Third, a key element in the advancement of our ability to understand and characterize shrimp viruses is the development of an *in vitro* cell culture system. Fourth, aquiculture pond effluents could be disinfected by through treatment with ozone and or permanganate to neutralize viruses. Solid waste could be incinerated. Both procedures serve to reduce the potential of accidental infection of wild populations through receiving waters.

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Charge to Panel Members- Shrimp Virus Peer Review

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

The focus of the management goal should include the aquiculture industry in order to reflect the true dimensions of the virus problem. Viral impacts to cultured shrimp throughout the world are known to be substantial and widespread, while evidence of impacts to wild shrimp populations is lacking.

Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquiculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

The assessment endpoints should reflect and emphasize the substantial potential risks of shrimp viruses to the aquiculture industry and should not emphasize risks that have no basis or have not been demonstrated.

It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestions.

A broader based input system would make the task of completing the proposed risk assessment unexceptably more difficult due to the extremely large data gaps that exist.

How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquiculture operations?

Information on viral infectivity and effects derived from laboratory or intensive aquiculture operations is not highly relevant to effects on wild populations. There are numerous examples in the literature of infection forced in the laboratory by, for example, injection of live virus that did not produce unusual moralities or is not exhibited in pond conditions. There is no good scientific evidence of any abnormal wild population declines due to viral effects although the aquaculture industry worldwide has a well known history of viral problems. For example, Ecuador's wild population of *P. vannarnei* continues to prosper although most of its 250,000 acres of shrimp ponds have been devastated by TSV and continue to discharge into the coastal waters. Furthermore, as Laramore (1997) reported, there was actually an increase in wild postlarvae over the next three years after TSV first appeared in aquiculture ponds in Honduras. The physiological stress and crowding of intensive aquaculture conditions may potentate the development and spread of disease that may not happen in the wild or less crowded conditions. The 1995 TSV outbreak that devastated South Carolina did not effect any impoundments that were stocked at lower densities, although they received seedstock from the same hatchery as those that exhibited disease, which supports the observation that crowding may significantly influence the expression of disease that may not be relevant in wild populations.

5. How likely is that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

It's very likely that both host and virus will adapt to coexistence. Historically, the exposure of populations to viral epidemics does not do permanent damage because of the development of immunity. For example,
we now have populations of *P.* stylirostris that are resistant to the IHHN virus and *P.vannamei* coexist with IHHN. Laramore (1 997) gives good evidence for the emergence of

a wild population of P.vannamei having increased resistance to the lethal effects of TSV. The tremendous fecundity of shrimp helps insure any potential negative environmental effects on populations survival over time.

6. How can the strong influences of both natural and non-viral anthropogenic factors on shrimp populations be separated from the risks associated with viral stressors?

Without additional data it's extremely difficult at present to separate the influence of natural and anthropogenic factors on shrimp populations from risks associated with viral stessors. Influences of individual stessors including those of combinations of stessors must be first quantified in controlled laboratory settings to demonstrate possible cause and effect (those factors that may predispose shrimp to disease.)

Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Human health effects from shrimp viruses can be ruled out since there is no evidence or suggestion of any effects to justify this.

Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

Identification techniques are only available for three of the four viruses that were focused on by the JSA workgroup and the complex nature of this testing may not allow for definitive conclusions to be made about the occurrence of viruses. For example, it is almost impossible to rule out the occurrence of viruses in large volumes of water or soil with these techniques.

U.S. aquiculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refue the importance of

aquiculture operations as a source for the virus?

There is no evidence from local wild populations to suggest that domestic aquiculture may be a source of virus. TSV that devastated Texas and South Carolina has not been identified in domestic wild populations. Also IHHN has not been identified in any wild populations. In South Carolina WSSV was first diagnosed Jan. 1997 in wild caught *P. setiferus,* and only later in Oct. 1997 showed up in one companies ponds.

It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

Despite the use of certified Specific Pathogen Free shrimp, the aquiculture industry continues to experience viral infections in which the sources may be of an external origin. There is no evidence to suggest that shrimp in aquiculture have infected wild populations, but there is some suggestion of wild populations infecting shrimp ponds. For example in South Carolina WSSV was identified first in wild stock prior to it appearing for the first time in an aquiculture growout pond (see above #9).

Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus.

As in aquiculture, there is no evidence of infectivity or declines in the wild population due to shrimp processing.

12. Should retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Shrimp viruses have been positively identified in imported farm-raised shrimp. Since retailers handle potentially contaminated imports and may distribute these, for example, as bait, they should receive additional evaluation as potential sources of exposure.

After considering the sources addressed in the shrimp virus report, what sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

An average of approximately one million pounds of farm raised-shrimp is imported into the domestic market daily. Probably a significant portion of this product goes directly to the retail and restaurant business without being touched by the processors.

Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

The usual processing temperatures that most shrimp feeds are subjected to are most probably sufficient to render any harmful virus inactive. I have consulted one viral expert who suggests we should not be comfortable at the lower processing temperatures mentioned (170-180 degrees F) without knowing the length of time the food is at this temperature during processing . He suggests hours rather than minutes.
However, if feed was a source of virus the effects probably would have shown up in the various diagnostic labs that must be using it in their challenge studies during bioassays.

How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline

of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquiculture into the wild in South America?)

The Gulf of California information originates from a Masters thesis and represents the best piece of epidemiological information available world-wide to suggest a link between introduced viruses and declines in wild shrimp populations. I have reviewed a translation of this and find no sound evidence that the decrease in' catch observed was due to IHHN. Further, there is no evidence that the IHHN found in wild stock originated in shrimp ponds - the opposite is just as likely. Decrease in catch followed a gradient with the lowest numbers found towards the blind northern end of the Gulf. With the atypical geography of the area there appears to be other stessors, such as pollution and low dissolved oxygen, that could have contributed to the decline observed. The decline observed in other species as well supports the possibility that other stessors may have influenced this decline. After the TSV outbreak in South America catch data indicates the population not only did not decrease but actually increased in later years .

There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?

It's difficult to assess the risk of pathogenic shrimp viruses in wild populations when there has been little monitoring or data to determine what is already present. For example, a pathogen already present in a wild population would represent a much lower risk to that particular wild stock than to Specific Pathogen Free shrimp stocked in aquiculture facilities that have no developed resistance.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Normal fluctuations occur in wild shrimp populations. There is already good documentation of catch data available for domestic species that currently show no unusual or unexplained declines in wild shrimp populations which is interpreted as a lack of evidence of a negative effect of possible introduced shrimp viruses. Population models, environmental data, and background levels of pathogenic shrimp viruses should be monitored in the future in order to spot and explain unusual population declines.

How important are potential viral effects on non-shrimp species?

Non-shrimp species are ecologically important however pathogenicity of viruses is usually species specific.

How will a comprehensive risk assessment contribute to management of shrimp virus problem, ie., will it add significantly to the information presently available?

It will not add significantly to information presently available. The best outcome of a tiered approach will be the organization of data needed to stimulate sound scientific information on viral epidemiology.

What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp populations models), and what would be the likely time from and cost?

A quantitative risk assessment with numerical estimates of the risks to shrimp populations would provide the best basis for making risk mitigation decisions. However, the extremely large data gaps at present will not support this. We must have a sound basis for such an

assessment that will require a large amount of critical additional research. Good population models must be developed and a determination must be made on what viral diseases, either native or introduced, are present in these populations.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

Yes, but treatment options associated with specific exposure scenarios would be valuable only if based on good new research data.

22. Summarize the critical research needs for completing such a risk assessment.

Critical initial research needs must include the following:

1. Development of definitive diagnostics

The lack of necessary tools as well as inconclusive and subjective tests make it difficult to test for possible pathogens.

2. Monitoring of wild populations

We need to know what is out there. We must determine what diseases are native to our populations and what the background levels are.

3. Monitoring of imports

Imports of farm-raised shrimp average approximately one million pounds each day, and based on volume, this potential source of viral introduction overwhelms all others. We need to know what's being brought in, how it's handled, and where it's going.

Anne Fairbrother

Management goals, assessment endpoints, and the conceptual model

1. *Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing and aquiculture operations.*

   This management goal is a little too narrow for the risk assessment, as it does not include the goal of keeping shrimp aquiculture virus-free as well. In fact, the viruses appear to have the potential to have a devastating effect on this industry, either through direct mortality of a year's worth of shrimp or through restrictions on exportation of the animals, regardless of their role in infecting wild populations. While the last clause of the goal statement may be interpreted to include this additional goal, it would be helpful to have it stated more explicitly, such as (bold text is suggested addition):

   Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. At/antic coastal *waters and the* ***shrimp aquiculture industry,*** *while minimizing possible* ***economic*** *impacts on shrimp importation, processing and aquiculture operations.*

2. The assessment endpoints should be modified for two reasons: 1) they are too broad for the current exercise and 2) they do not include the aquiculture industry (see comment #l). In regard to the first point, the assessment endpoints suggest that the risk assessment will look at all possible causes of changes in survival, growth and reproduction of wild penaeid shrimp, including indirect effects of ecological structure and function. In reality, the current risk assessment is focused only on assessing the risk that introduced viruses pose to the wild shrimp populations and shrimp aquiculture. Therefore, the assessment endpoints for this risk assessment should be narrowed; later, they can be expanded to examine ail other potential environmental stressors and their interactions.

   It is perfectly acceptable to ask a narrowly focused risk question, particularly in a case such as this. If it is determined that the nonindigenous viruses do not pose a risk to shrimp, then there is no need to go any further. If, on the other hand, it is determined that there is a high probability that the viruses could severely reduce the wild shrimp populations or make aquiculture economically infeasible, then there may be a reason to look at all the potential stressors on shrimp and determine the

relative risk from viruses as compared to other environmental degradation processes.

In particular, the second assessment endpoint listed on page 18 of the report could be deleted. An additional assessment endpoint should be added to address the concern that introduced shrimp viruses may have a broad host range and affect other marine organisms (e.g., clams or fish) as well. Suggested wording would be:

Maintenance of viable populations and communities of marine organisms other than penaeid shrimp, free of virus-induced effects.'

Note that what this does is to remove the endpoint of ecological structure and function and specify the more narrow assessment goal of the maintenance of populations of marine organisms. This allows the current risk assessment to be focused on effects of introduced pathogens, and does not imply that the assessment will include such things as coastal development, water diversion projects, etc.

Finally, an additional endpoint should be added to address the aquiculture issues, for example:

Economic viability of the shrimp aquiculture and processing operations.

3. The above comments suggest that I believe that it is useful to keep the scope of the current risk assessment narrowly focused on the question of the potential risk of introduced virus. There may be a need to do a comprehensive risk assessment at some point, but that does not preclude asking this particular question about the potential effects of viral introductions.

Viral stressors and factors regulating shrimp populations

4. Information on infectivity and effects of viruses derived from laboratory or aquiculture operations is very relevant to the *potential* for effects to occur in wild populations. In fact, it is only through laboratory studies that Koch's postulates can be fulfilled, thus proving that a particular pathogen is the causative agent of an observed disease. Most of the known diseases have been studied in the laboratory at some time. Lab studies are particularly useful for establishing potential host range (i.e., susceptibility of various species to the virus) and an idea of how much virus must be present to initiate an effect in an exposed organism.

Of course, laboratory studies only identify the *potential* for effects in wild populations, as they do not account for all the exposure factors. Assuming that all environmental conditions are exactly the same as the laboratory, one could predict with a great deal of certainty what the effects would be. Given that this is never the case, uncertainty in extrapolating lab results to the probability that effects will occur in the field increases. However, laboratory studies also can provide information that can be used to extrapolate lab results to field situations, such as the range of environmental conditions tolerated by a virus (e.g., PH, temperature, water quality), the transmissibility of the agent (e.g., how close together do hosts need to be in order to become infected), how the agent passes from one generation of hosts to another (vectors, transovarial transmission, water dispersal, etc.).

5. I am not qualified to speak authoritatively about development of immunity to viral infections in shrimp, as I am not familiar with shrimp immunology. If they are similar to shellfish (e.g., clams), then they would have the capability to develop immunity (also known as "resistance"), provided the virus is not 100% lethal with a high transmissibility rate. It is to the advantage of both the host and the virus to become more commensalistic through time, i.e., for the host to develop resistance and for the virus to become less virulent. There are numerous examples of this occurring in vertebrates, the most well-known being the introduction of myxomatosis virus to Australian rabbits. The one notable example where this has not occurred is rabies, which is nearly always fatal to the host so natural immunity (i.e., development of antibodies) does not occur. However, it is noteworthy that the virus has adapted to this by initiating a behavior prior to death (e.g., salivation for virus shedding, aggression, and biting) that nearly guarantees transmission should another susceptible host be nearby. Rabies also has a relatively low transmission rate since it requires direct contact of an infected and susceptible host.

6. The rk from viral stressors should first be assessed as if the virus was the only stressor present. Then, modifying factors would be added that could potentially change the host-virus interaction. For example, changes in hydrology of the aquiculture system of the nursery marshes, changes in density of the shrimp due to harvesting or natural factors, etc. The viral risk to the shrimp under these modifying

conditions then could be assessed. If one wishes to compare the risk from viruses to the risk from other environmental stressors (i.e., do a comparative risk assessment of viral risk versus risk of overharvesting or risk of reduction of nursery areas or risk from bacteria and parasites, etc.), then each of the potential stressors would need to be assessed both individually and in appropriate combinations as modifying factors of each other. This would be a very long and intricate process, but could be done.

7. There was insufficient information provided in the report to rule out potential human health effects from all the viruses. The white spot syndrome virus (WSSV) is a Baculovirus, a virus group which has no known vertebrate hosts (non-occluded baculoviruses such as WSSV cannot tolerate the acidity of the GI tract or the relatively high body temperature of vertebrates). Therefore, this virus could be ruled out as a potential human pathogen. Two of the virus groups have known human pathogens: polio belongs to the picomavirus group along with taura syndrome virus (TSV) and rabies is a rhabdovirus similar to yellow head virus syndrom (YHV). Infectious hypoderma and hematopoietic necrosis virus (IHHNV) is a parvovirus, a group that primarily, infects' animals (e..g., canine parvovirus or feline panleukopenia). These groups also include viruses pathogenic to domestic and wild animals, some of which have great economic concerns, should they affect livestock. Note, in particular, that there are 5 pathogenic rhabdoviruses in fish, affecting rainbow trout, carp and pike in Europe, salmonids in the Pacific Northwestern US and the American eel. Therefore, there should be discussion about potential pathogenicity in *any* vertebrate, particularly when discussing the possibility of birds or other animals to act as vectors of transmission.

It should be noted, however, that many of the viruses in these three groups have restricted host ranges, so there is an equal possibility that humans and other vertebrates would *not* be susceptible to the viruses. None of the viruses in these groups have been known to infect *both* vertebrates and invertebrates (the only viruses that do this routinely are the arboviruses, a group comprised mainly of encephalitic viruses that infect and are transmitted by arthropod vectors), so the probability of human infection is remote. However, until more information is provided about host range and environmental tolerances of these viruses, it is not possible to

make *a priori* predictions about transpacific susceptibility. Some simple cell culture laboratory studies could provide a great deal of reassurance in this regard, while simultaneously providing information about the environmental persistence of these viruses.

8. The report states that a gene probe is available from commercial sources for IHHNV and WSSV, which would suggest that reliable identification methods are available for drawing definitive conclusions about the occurrence of these viruses in shrimp, other organisms, or the environment. The other two viruses do not have such reliable identification tools, and epidemiology must rely on bioassays or electron microscopy. While these more traditional methods can provide a great deal of information, they are neither as definitive nor as quick as a gene probe.

Viral pathways and sources

Aquiculture

1. The report identifies several potential routes for introduction of exogenous pathogens into the populations of wild shrimp in the Gulf of Mexico or the Atlantic Ocean off the southeastern US coast. These were detailed in Figure 8 of the report and include: water discharges from aquiculture ponds; sludge dumping from aquiculture ponds; escape of infected shrimp; spills or losses during transport to the shrimp processing facilities; or through use of infected shrimp as bait. Page 25 provides further discussion of shrimp phenology that appears to support the possibility of aquiculture to wild shrimp virus transfer. However, no data were presented that would substantiate a conclusion about the actuality of such a transfer. What would be needed would be isolation of similar viruses from an aquiculture facility and a geographically connected wild shrimp population. Using gene probe technology, it should be possible to determine if the viruses were, indeed, the same agent. Without such information, the role of aquaculture in infection of wild shrimp remains speculative.

   It should also be noted that infection of a local population of shrimp as a result of aquiculture practices might or might not result in pathogenic infections of the entire population through the Gulf. More information is required about pathogenesis,

carrier states, and transmissibility before such conclusions could be drawn. For example, if the virus is very pathogenic, it may wipe out the local population before it has time to come in contact with other subpopulations. If the virus persists in the environment of the local nursery marsh, any shrimp coming in to breed in subsequent years may be infected and die, making the marsh unsuitable to continued shrimp production. But the population as a whole might remain uninfected.

2. The observation that domesticated animals rarely infect wild animals while the converse frequently happens is not true. Avian cholera (*Pasteurella multocida)* is a devastating disease of wild waterfowl, killing as many as hundreds of thousands every year in North America. This disease was introduced to waterfowl from the poultry industry in Texas in the 1940s. Duck viral enteritis, a herpesvirus, was introduced to North American waterfowl from the domestic duck industry on Long Island, NY in the 1960s. Brucellosis *(Bruce//a abortus, B. canis,* and *B. suis)* was introduced to the American bison, various wild cervids (deer and elk), wild canines' (coyotes), and wild pigs from domestic livestock. Tuberculosis *(Mycobacterium bovis)* occurs in many species of cervids and bovids where they come in contact with domestic livestock. Mycoplasmas (e.g., *Mycoplasma gallepticum* or *M. synovium*) are picked up by wild turkeys that intermingle with domestic turkey flocks. There are many, many other such examples of domestic animal to wildlife transfers of disease agents.

   Transmission of diseases from wild animals to domestic livestock or pets is less well documented. Rabies and rinderpest (a paramyxovirus) are perhaps the best known examples of wild animal reservoirs with direct transmission to domestic animals. Foot-and-mouth disease (a picornavirus) and other vesicular diseases (in the rhabdovirus group) may be endemic in wild hoofed stock in Africa, providing a reservoir for infection of range cattle. Myxomatosis virus (an arbovirus) is endemic in wild rabbits in California, and occasionally infects domestic herds. Other organisms that persist well in the environment may infect both wild and domestic animals equally, and include diseases such as anthrax, leptospirosis, and tularemia. Other groups of organisms that cycle regularly between wild and domestic animals are the arboviruses and rickettsial diseases that are maintained in wild vertebrate

hosts with transmission through arthropod vectors. Occasional epidemics of disease occur in domestic livestock or humans, including such devastating diseases as yellow fever and dengue fever. Other agents have a lower, more endemic pattern such as Lyme disease or Rocky Mountain spotted fever.

In sum, there is ample evidence that domestic animals (e.g., shrimp aquiculture) may infect wild animals (e.g., wild shrimp populations) should there be appropriate co-occurrence of infected and susceptible populations or contamination of the environment.

Shrimp processing

3. As with the shrimp aquiculture industry, the shrimp processing industry has the *potential* to discharge virus-contaminated materials into waters inhabited by wild shrimp, particularly due to the practice of receiving shrimp from other countries that harvested shrimp during the early states of a disease outbreak (page 26 of the report). Section 3.7.1 of the report describes what is known about infection of wild shrimp by IHHNV, TSV, WSSV, and YHV. Based on this information, there is little evidence to either support or refute the hypothesis that the processing industry is a source of infection.

4. Whether or not retailers who distribute (rather than ,process) shrimp products should receive additional evaluation as potential sources of exposure to wild shrimp depends upon whether they discharge any shrimp products to marshes, shorelines, or oceans. As they likely do not, it would not seem necessary to investigate them further.

Other potential sources and pathways

5. The most critical additional sources and pathways of infection of wild shrimp and aquiculture include: bait shrimp and ballast water discharges. Research and display aquaria would have similar issues to aquiculture and so need not be considered separately. Non-shrimp translocated animals (e.g., shellfish, crabs, etc.) may be important, but since we do not know anything about host range of the viruses it would be difficult to evaluate this pathway. Indeed, ballast water discharge includes the potential for translocation of infected organisms as well as contaminated water. Vector transport by nonsusceptible hosts (e.g., birds) has a low probability. Natural

spread should be considered, again within the context of little knowledge about environmental persistence or host transmission rates.

6. There is no information presented in the report about the composition of manufactured shrimp feed or the temperature to which it is subjected. However, if the temperature is high (>100°C), then it is likely that the viruses would be killed.

Stressor effects

7. The available evidence concerning the effects of introduced viruses on wild shrimp populations should be interpreted with caution. The role of IHHNV in the decline of shrimp population in the 1980's in the Gulf of California is speculative - correlation does not equal cause-and-effect. I believe the points made on pages 42 and 43 of the report about why viruses (and related effects) have not been detected in the U.S. wild stocks is right on target. Collection of TSV-infected shrimp from near-shore or off-shore fisheries in Ecuador, El Salvador, and the southern Mexican state of Chiapas suggests that the virus might exist in these free-living populations, but insufficient data are presented in the report to determine if this is a conclusive statement.

8. The limited data on background levels of pathogenic shrimp viruses in wild population in U.S. waters must be evaluated cautiously. Pages 42-43 of the report suggest that we really do not know whether or not these viruses currently are present. Until more information is made available, the risk assessment should assume that they are not endemic as a worse-case scenario.

9. Shrimp population numbers suggests that there are forces in the environment that can control shrimp populations. Correlational studies can suggest what some of these factors might be. For example, comparing climate cycles, hurricane incidence rate, ocean temperatures, harvest rates, or known viral introductions with population numbers can suggest which one(s) may have the greatest potential for effect. In order to quantitatively model the relationship of viruses and shrimp population numbers, information on the age-class specific infectivity rate, transmission rate, mortality rate, and immunity rate needs to be made available, none of which appear to be very well known.

10. The importance of potential viral effects on non-shrimp species is not known, as there is no information on whether or not other species are susceptible to these viruses. [f they are, then the same suite of information outlined in the previous comment would need to be understood for these species as well, in order to derive a definitive answer. However, the evolutionary pattern appears to be that a newly introduced pathogen may be extremely virulent initially, killing a large percentage of the host population. Eventually, either one of two outcomes occurs: the host population is completely destroyed (rare occurrence) or the host-virus association is modulated towards co-adaptation, with the host becoming less susceptible and the virus becoming less pathogenic. The population may, however, become stabilized at a lower density that previously. Both the initial population depression and the subsequent reduced equilibrium numbers may put an industry, such as the wild shrimp or shellfish harvesters, at an economic disadvantage.

Comprehensive risk assessment and research needs

11 A comprehensive risk assessment will not add to the available information. The risk assessment process uses information and synthesizes it to generate a risk statement, it does not develop new information. In the process, however, information gaps are identified and new information may be gathered prior to a second iteration of the risk assessment. This helps to focus research into areas that will immediately result in a reduction in the uncertainty associated with the risk prediction. Therefore, the risk assessment process can be very useful in identifying data gaps and prioritizing research needs.

12. Following the qualitative risk assessment, a quantitative risk assessment using shrimp models could be done but on/y if additional information about viral pathogenesis (transmission, immunity, mortality rates; see above comment) is provided. Additional information that would be required is persistence of the virus under various environmental conditions.

13. Risk reduction potential of various treatment options should eventually be considered, once more information is available about the virus (see previous comment).

14. Critical research needs for conducting a quantitative risk assessment include (at a minimum): viral pathogenesis; viral resistance/susceptibility to environmental conditions; endemicity of virus in U.S. coastal populations, interspecific susceptibility and transmissibility; identification of virus in possible vectors and sources. The list of data gaps presented on page 49-50 is fairly complete. A reasonable first step towards assessing risk would be a well-conducted survey of the U.S. coastal shrimp populations to determine if these (or other) pathogenic viruses are endemic in the wild populations. If they are, the risk from further introductions might be considerably less than if the populations are naive.

William Fisher

1. The management goal falls short by focusing only on four shrimp viruses, which are of current concern, but may only be the tip of the iceberg. This does not account for other micro-organisms and small eukaryotes (such as isopods), that are parasites, pathogens, commensals, and symbionts of imported shrimp, regardless of whether they are imported for aquiculture or food processing. Nor does it account for populations of indigenous organisms that can be exacerbated by the high-density conditions of aquiculture. Focus on the four current viruses assumes that no other viruses (either latent or undetected) and no other organisms will disrupt the wild populations of shrimp. South Carolina apparently monitors for at least nine different organisms . . . where are all of those included in this management goal?

2. A third assessment endpoint should focus on ecological aspects NOT NECESSARILY related to wild shrimp populations and harvest. Society has many different values for estuarine resources and these require an estuarine infrastructure (= integrity) that sustains those values. if any organism brought into the estuary alters that infrastructure, then values other than wild shrimp harvests may suffer. For example, imported penaeid shrimp may carry organisms that are not harmful to wild penaeid populations, but do impact grass shrimp populations. The many commercial fish species that rely on grass shrimp during their estuarine nursery life stages would be affected, as would the harvests of these fish; additional social values at risk.

3. It is important to remember that wild shrimp harvesting techniques are very destructive to coastal habitats and several different marine organisms, and that one important value of aquiculture is the potential to develop non-destructive, or minimal impact, food production capabilities. Regardless of how important it is to reduce the impact of wild shrimp harvests, this issue does not help to focus on risks and consequences of nonindigenous introductions.

4. Obviously any extrapolations must be verified. Certainly the highly contagious conditions of high-density, high nutrient aquiculture will not reflect a natural condition.

5. Over time, and assuming that there is reasonable genetic vitality and cross-breeding, this is a reasonable scenario. But there are a minimum of 4 (probably many more to appear in the future) organisms that may have to go through this selection process and mortalities will be high during the period of development of resistance. Crustacea are not vertebrates, so they do not have antibodies to provide specificity and memory in an immune response. Protection, or resistance, usually comes from selection pressure exerted over many generations that ultimately allows host and parasite to reach an equilibrium that is not as destructive to the host.

6. It is usually difficult to distinguish the actions of single stressors when the occurrence of disease requires a suitable juxtaposition of host, parasite and environmental conditions (Snieszko paradigm). However, if non-indigenous viruses are associated with disease, then their introduction should be considered a highly significant factor.

7. Zoonoses are rare. In most cases, parasites survive because of their ability to use a unique or unused resource; consequently they develop close associations of dependence on specific host populations.

8. No response.

9. The fact that there have not been reports of massive epizootics (or only one reported) is not sufficient to suggest that local wild populations have not been infected. In the wild, infected shrimp may not die, may not die immediately, or may not die *en masse to* be detected. It may be that responses are less acute than observed in high density and high nutrient conditions of aquiculture. Or that on or more of these viruses is not expressed unless environmental conditions are met. Therefore, only specific diagnostic techniques for the presence of the virus should be accepted as a measure of exposure (infection). Information developed using such techniques would also have to be based in a defendable monitoring effort, with appropriate frequency and timing of samples. Also, if viruses are detected near an aquiculture facility, this is not sufficient evidence to proclaim it the source; however, such a finding should instigate an investigation.

10. It's hard to think of shrimp in aquiculture as "domesticated". Mostly they are offspring of wild shrimp that have been penned and repeatedly spawned. Programs like the SPF broodstock development may begin to move shrimp toward natural and artificial selection that leads to domestication. Nonetheless, the question is valid. Unfortunately, the ease and ability to monitor diseases of domestic "penned" populations far outstrips our ability to monitor wildlife diseases, so this influences our observations of the rate of occurrence in or out of pens and corrals. It is possible that a wildlife disease expert may have many examples of agricultural plants or animals creating major impacts on natural populations.

11. See #9

12. Yes, depending on the status of the product (boiled? raw?).

13. There is some confusion here since it is not apparent that bait shrimp come from foreign sources, so the occurrence of these "exotic" (presumably meaning non-indigenous) viruses should not be a concern in bait shrimp. However, indigenous viruses and other organisms should certainly be a concern (see #1). Other concerns (ballast, research display, other translocated animals) maybe valid concerns, but the potential for large inoculations is less. The larger the inoculation, the greater opportunity to become established.

14. No response.

15. From the summaries presented, it appears that they can only be interpreted as *potential evidence.* Without better documentation, they cannot be used to demonstrate source, direct cause, effect, lack of long-term effect, or development of resistance.

16. This lack of information is not particularly relevant if it refers to background levels of indigenous viruses, since the primary concern here is non-indigenous viruses. If we simply do not know whether these 'exotic" viruses already exist in U. S. wild populations, then the lack of information becomes very important. If the viruses are

indigenous, then the wild shrimp population may not be as 'immunologically' naive as otherwise suspected and the primary concern should shift to the potential impact of additional dosage or stresses issuing from anthropogenic activities. This question seems to infer that effects of introduced organisms may be altered by existing disease conditions; if so, this is corollary, and not primary, to protecting wild shrimp populations from introduced viruses (we don't need to know how many people have pneumonia to protect the population from a new strain of pneumonia, or from influenza).

17. The most obvious scenario is massive mortalities of wild shrimp populations with clear evidence of viral infection from a previously unreported (and presumably nonindigenous) virus. Increased /decreased presence of virus in wild populations can also be used. Stock assessments are much more difficult to interpret.

18. Probably shrimp viral effects are not very great on non-shrimp species, but a major shortcoming of the report is the lack of concern over non-penaeid shrimp species. For example, grass shrimp (Palaemonidae) include species that are dominant (biomass) in many southeastern estuarine systems and serve vital ecological roles in nutrient cycling (detritovores) and as prey for important commercial and non-commercial fish species during their early developmental stages. Major losses of these organisms would severely impact many important sport and commercial fisheries and undermine the existing estuarine infrastructure. A second issue that this question raises is the importation of organisms unrelated to shrimp — microorganisms or small eukaryotes that are commensally or inadvertently associated with shrimp, on the gills or in the digestive glands, that are potentially harmful to other native organisms.

19. It should organize the information and create the dialogue to qualify the information available,

20. A conservative tiered approach. It would be unlikely to resolve many of the issues in a short period of time.

21. Yes, but not limited to treatment options — include prevention options such as location of aquaculture and processing plants away from estuaries.

22. Research needs are to determine:

- What organisms (virus, bacteria, fungi, eukaryotes, etc.) are imported with any foreign shrimp, whether for aquiculture or processing.
- Which of these survive and are present in effluent from aquiculture or processing
- Which surviving organisms are capable of infecting, infesting or associating with wild shrimp or other estuarine/ coastal inhabitants (particularly other shrimp species).
- What the consequences of such an association are on the organism, population and community.

Corollary question:

How do high-density, high-nutrient conditions of aquiculture exacerbate the proliferation and contagion of resting or latent microorganisms, indigenous and non-indigenous.

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Premeeting Comments: JSA Shrimp Virus Report

Management goals assessment endpoints and the conceptual model

1. The management goal should reflect the full scope of the problem. As such it is adequate if the scope is limited to estimating the risks to wild shrimp populations. Should the scope be widened to include non-shrimp species then the goal will need to be modified to reflect that change

2. My perspective has always been that the conceptual model should capture the full spectrum of probable risks and thus include a suite of assessment endpoints. This approach requires that one must include all the drivers, stressors, and possible interactions of importance operating on the assessment endpoints. If one accepts this strategy then if there is a probable risk to non-shrimp organisms and the larger estuarine ecological system then the suite of assessment endpoints should be expanded. It is important in conducting risk assessments to assure that potentially important interactions are identified and points of connectivity between endpoints or systems are represented. However, all risks within the conceptual model are not likely to have equal probabilities, that is, some are more important than others. Thus the risk assessor must rank the probable risks and provide a rationale for the decision to examine one risk rather than another. In this case, I would suggest that the full conceptual model be constructed and the probable risks weighted. This conveys the ideas that all risks were considered but these were the most important and selected for further study. Unless there is compelling evidence to suggest that there are no non-target species/system risks, that there are no plausible interactions with and connectivity to other systems then a broadening of the scope of the assessment should be considered

3. This follows logically from #2 above. Broadening the scope of the conceptual model logically requires addressing other drivers or sources of stress to the system. A point of clarification on terminology - a stressor must co-occur in space and time with the ecological endpoint/receptor - things like landuse changes, production methods are generally not stressors to aquatic systems rather they are drivers, sources, or agents that lead to stress. However, as part of expanding the conceptual model it will likely be necessary to expand the stressors and to identify specific interactions that may be important

Viral Stressors and factors regulating shrimp populations

4. The translation of laboratory to field is an issue that is common to most of the research we conduct. In general, laboratory studies permit the establishment of principles and pathways of causality under controlled conditions. Laboratory studies do establish the likelihood of realizing a particular stressor-response relationship often for optimum conditions. Translation to the field depends on the degree to which the laboratory conditions are realized in the field. If the laboratory study tests a range of response for what are considered critical variables then the likelihood of transference is enhanced. If the laboratory study is poorly designed then the uncertainty associated with transferring this data to the field would be so great as to be meaningless.

5. I have no comment on this question

6. Assigning relative importance of risks from multiple stressors is a generic problem in most risk assessments. Typically one looks for biological/ecological responses or markers that are specific and diagnostic for a particular stressor. If this relationship can be established the response must then be scaled to effects at the population level. In the case of shrimp, there are natural climatic factors influencing shrimp stocks, there is fishing pressures, as well as disease to name a couple. If there is a biological probe that can determine the proportion of a shrimp population that are infected it could be treated as mortality and then projected to a loss in population size.

7. This is certainly an important question and one for which I suspect there is insufficient evidence upon which to make a judgement with low uncertainty. This problem of animal diseases moving to humans seems to be becoming more and more of a concern with several incidence being documented lately. I suggest that the human health issue is never one that can be dismissed nor should it be without substantial evidence.

8. I have no knowledge regarding this question. It is important to have as many reliable diagnostic tools as possible for screening infection. Having such a tool would be invaluable for monitoring wild and cultured shrimp stocks as well as various steps in the process stream and field exposure pathways. I would suggest that this is an important data gap and research need.

Viral Pathways and sources

Aquiculture

9. I have no experience with this topic. However, having a molecular probe or marker for the various virus types certainly would help to address this question.

10. Again this is not my field but a couple of questions come to mind, What is the evidence supporting the statement that it is unusual for domesticated stocks to infect wild animal populations and vice versa. Cultured shrimp are not really domesticated in the true sense of the term are they? More importantly if animal virus are now moving to human hosts with increasing regularity why should one not suspect that viruses from cultured populations can infect wild stocks all factors being equal?

Shrimp processing

11. This is an area with which I have no specific experience. However, I suspect monitoring

and experiments have been conducted that will address this question. Again it seems to be a question of having the appropriate monitoring methods for reliably detecting the viruses in the wild from those processed from cultured populations and being able to discriminate the origin of viruses from different sources (assuming they have unique markers).

12. I think this question falls more within the human health risk arena. From a health perspective shouldn't this be included within the rubric of "seafood safety" similar to concerns over bacterial contamination, biotoxins, and organic and metal contaminants. I think that the human health issue is particularly important for the retailing industry.

Other potential sources and pathways

13. No comment

14. The answer to this question depends on the process used and the viability of the virus under those conditions. If elevated temperatures (e.g., pasteurization of some type) could be used in the process without damaging the product then that would be a simple and inexpensive control mechanism that could be used in most countries.

Stressor effects

15. Though not familiar with the data from this field one general approach is commission a critical review of the data by a group of independent scientists.

16. The absence of natural background levels of shrimp virus pose at least two problem for the risk assessor. First, is the size of the natural source of the virus and thus its potential for causing effects. Without knowing background levels it is difficult to interpret what could be considered "normal" and whether management actions are having an affect. By knowing the controlling factors and the range of natural variability the risk assessor can then assess the efficacy or risk reduction efforts. Further, knowing the natural variability provides insight into potential impacts to the population. The incidence can be used as a variable in a population model to project the range of expected populations as a function infection

frequency. Thus when some anecdotal evidence is reported for the population or catch one can examine it within a context of the natural variability and degree of infection.

17. See #16 above

18. I do not know the answer to this question but suggest that it should be considered important until evidence proves otherwise. As discussed above under conceptual models, this is an important element that should be included in the conceptual model and as part of a comprehensive risk assessment.

Comprehensive risk assessment and research needs

19. A comprehensive risk assessment will put some real numbers on what now seems to be expert judgement. There is nothing wrong with the latter and in fact it is often as far as the risk assessor can go given available information. However, it doesn't treat uncertainty which is important for decision-making. At frost glance, it may not add significantly to current information but it will put all the information within a systematic framework where it can be analyzed and evaluated. Further it will quickly identify critical data needs both in terms of quality and quantity. If nothing else it will tell you what you know and don't know and how confident you are with what you know and don't know.

20. I'd like to suggest that a full simulation model rather than just a shrimp population model. I say this because I don't know any other way to capture the full suite of variables and their interactions including multiple drivers, stressors, and modifying variables. Further the assessment endpoints should not be limited to only the shrimp population but should include other types of endpoints that could not be ascertained from just a shrimp model. However, in lieu of having a simulation model, the shrimp model can be used to test a variety of hypotheses or potential scenarios as long as one recognizes it limitations.

21. Absolutely. I'm a strong proponent of scenario-consequence analysis because it allows the risk assessor to play "what if games" without having to have every piece of information and know every uncertainty. In addition if scenario analyses are coupled with sensitively

analysis valuable information is revealed that helps identify the most important variables contributing to the risks. This information can then be used to allocate research on obtaining those pieces of information that are most important and which contribute the most to reducing uncertainty.

22. I'd like to suggest that this is one of the outputs from the workshop.

Rebecca Golburg

Comments for the Shrimp Virus Peer Review Workshop, January 7-8, 1997

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I have structured the following comments as answers to questions in the "Charge to Panel Members" for the Shrimp Virus Peer Review Meeting, although I do not answer every question. In some cases I have drafted cornments to answer two or more related questions.

My comments were prepared with input from Pam Baker, who works for the Environmental Defense Fund (EDF) in Texas, and from Dr. Cristina Tirade. My comments also draw on EDF's August 29, 1997, comments to the National Marine Fisheries Service concerning the JSA shrimp virus report. The August comments were prepared by Pam Baker, Dr. Doug Rader of EDF's North Carolina office, and me.

Questions:

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquiculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

18. How important are potential viral effects on non-shrimp species?

Answer/comment:

The management goal of the JSA report (p. 14) is generally appropriate. Nevertheless, I suggest the following additions (underlined), so that the management goal reads:

Prevent the establishment of new disease-causing viruses in wild populations of shrimp and other susceptible organisms in the Gulf of Mexico and southeastern US Atlantic waters while minimizing possible economic impacts on shrimp importation, processing, and aquiculture operations.

Add a statement following the goal stating that "When feasible, source reduction aproaches will be the preferred methods for achieving the management goal."

The reasons for these three underlined changes are discussed below.

First, the goal should be broadened to include wild populations of susceptible organisms other than shrimp. The JSA report makes clear that some shrimp viruses may infect a range of invertebrates other than shrimp. Introduction of new shrimp viruses could therefore potentially lead to decreases in populations of a variety of organisms and even undermine marine food webs. The management goal should reflect these ecological concerns, as well as largely economic concerns about shrimp populations. Consideration of organisms other than shrimp may also be important to economic objectives. The health of marine food webs affects the health of fisheries and thus effects of new shrimp viruses on organisms other than shrimp could cause economic harm.

A challenge, of course, is to keep the risk assessment manageable: It is not a simple matter to fully assess the risks to marine ecosystems of new shrimp viruses. Nevertheless, in the short-term, the qualitative risk assessment could consider the limited information available about the host ranges of various shrimp viruses, and lay out the potential range of consequences establishment of new shrimp viruses could have for marine ecosystems. Over the longer term, research to better delineate the host ranges of new shrimp viruses should be a priority. Such additional information will almost certainly be necessary to judge the likely effects of shrimp viruses on marine ecosystems.

Second, the potential economic impacts should be minimized from any actions to prevent the establishment of shrimp viruses. Given the potentially devastating impacts of new shrimp viruses, the federal government should not shy away from working with or requiring the shrimp importation, processing, and aquiculture industries to make any changes necessary to protect wild populations of shrimp and other organisms. The management goal should be to keep the costs of any necessary changes as low as possible.

Third, source reduction should be acknowledged as the preferred means of addressing threats from new shrimp viruses. Over the past several decades, the strategic foundation for pollution control has evolved so that there is now a recognized spectrum of approaches to managing pollutants. The most preferred of these approaches is to prevent or reduce the production of pollutants in the first place. In decreasing order of preference, other approaches are to recycle and reuse wastes, waste treatment, and disposal of wastes in the environment. This ranking was written into law by the US Congress in 1990 under the Federal Pollution Prevention Act.' Although this spectrum of approaches most often is applied to manufacturing industries, it is applicable to terrestrial agriculture (Hoppin et al. 1997), and should be applicable to shrimp aquiculture.

Many shrimp aquaculture operations, particularly outside the United States, have poor environmental and other practices that lead to disease outbreaks on farms (for

'42 U. S. C. Sec. 13101-13109

example, Clay 1996; Gujja and Finger-Stich 1996; Hopkins et al. 1995). These disease outbreaks are the root cause of current threats to the US shrimp fishery and US coastal ecosystems from new shrimp viruses.

Source reduction -- preventing imported and domestic farmed shrimp from becoming infected by new viruses -- should be the most preferred approach to preventing the establishment of new shrimp viruses in wild population of shrimp and other organisms in the United States. In a pollution prevention framework such an approach is clearly preferable to say, trying to stop introductions of new shrimp viruses by requiring on complete disinfection of effluents from coastal shrimp processing plants in the southeastern United States. Admittedly, there are hurdles to filly implementing a source reduction approach, and waste treatment approaches are likely to also be necessary. All the same, the management goal should make clear that risk management approaches to address threats from new shrimp viruses should be developed within a source reduction framework.

Question:

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that is should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

Answer/comment:

The scope of the proposed risk assessment should not be broadened to consider alternative land uses and seafood production methods (beyond alternative shrimp farming practices). It is possible to draw linkages from just about every environmental problem to a range of other problems and circumstances in our society. However, progress on any one environmental problem usually depends on sufficiently narrowing the scope of the issues considered in order to make the problem tractable. Broadening the scope of the risk assessment for new shrimp viruses to include land use and general seafood production issues would do just the opposite -- making the risk assessment process lengthy and possibly intractable. Particularly given the urgency of the potential threat from shrimp viruses, it would not be prudent to broaden the scope of the risk assessment to consider these issues.

Question:

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquiculture operations?

Answer/comment:

Laboratory results can provide valuable information about viruses. Lab results concerning mode of transmission, virus viability, and the capability of survivors to become carriers are highly relevant to the risks that viruses pose to wild populations. Results from the aggressive challenges with a particular virus are valuable indicators of the relative susceptibility or resistance of wild individuals or populations to a the virus.

Nevertheless, a rule of thumb across many fields of biology is that pathogens more readily infect organisms under experimental conditions (in a laboratory, greenhouse, etc.) than they do in nature. Laboratory data concerning the effects of viruses provides an useful evidence about the potential effects of viruses on wild populations, but does not always predict how diseases affect wild populations.

There are several reasons why the itfectivity and mortality observed in experimental infections of shrimp are likely to be more severe than what would probably be in the wild. For example, researchers often try to maximize the odds of infection by injecting shrimp with purified viral suspension or by feeding shrimp a diet with large amounts of infected material. In addition, lab animals are generally not subject to predation by other species. In contrast, wild animals weak from illness tend to suffer high rates of predation, reducing the chance that diseased individuals will transmit their infections.

Intensive shrimp aquaculture operations also have characteristics that tend to promote the spread of disease. High stocking densities and environmental and handling stresses in intensive systems increase the susceptibility of shrimp to disease and the chance that they will become infected. For example, shrimp in intensive systems are often continuously exposed to virus-laden water. Infected animals tend to suffer high cannibalism rates, spreading disease. Some viruses may be vertically transmitted in spawning tanks. In contrast, the odds of horizontal transmission of viruses is lower in the wild, because populations are relatively sparse and cannibalism rates are relatively low.

Question:

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

Answer/comment:

It is difficult to speculate whether the exposure of wild shrimp populations to viral diseases would lead to the development of immunity and reduced effects on population survival. Information about immune mechanisms in shrimp is very limited and mostly concerns the response to commercial “immunostimulants” (cell-wall components from fungi or bacteria) and *Vibrio* vaccines, which do not necessarily provide complete or long-term protection against diseases. The relative protection provided by these vaccines may result from a general stimulation of cellular defense mechanisms rather than the

development of immunity. Crustacea do not display long term specific immunological memory because they do not express specific antibodies (immunoglobulins).

Nevertheless, some scientific literature suggests that shrimp survivors of at least some viral infections are more resistant to challenges with that viral agent than shrimp that were not previously exposed to the virus. For example, Erickson et. al (1997) reported that *P. setiferus* and *P. vannamei* TSV survivors were relatively unaffected by a challenge with TSV (90% and 45% of individuals of each species, respectively, survived), while *P. vannemei* that were not previously infected were very sensitive to the challenge (only 7.5% of individuals survived).

In the wild, natural selection may have a greater effect than immunological mechanisms on reducing mortality rates from viruses. When virus is present, individuals with genetically-based resistance to a virus will tend to have more offspring that survive and reproduce than relatively susceptible individuals. Resistant genotypes may thus come to dominate a population. Of course, viruses also evolve, and they may mutate to become able to harm what were once relatively resistant genotypes. Of note, both YHV and TSV are RNA viruses, which are regarded as having rapid rates of evolution.

Question:

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

Answer/comment:

Ecologists measure the effects of various factors on population density by performing controlled experiments. The effects of biological factors (for example, predators) are measured by excluding these organisms from some experimental plots. Experiments typically employ a factorial design if more than one factor is being studied.

Experiments to measure the effects of various factors, such as viruses, on shrimp populations would likely be impossible to perform with wild shrimp populations. However, small-scale lab experiments looking at, say, the effects of temperature and viral infection on fecundity may provide some clues to the relative importance of various factors.

Question:

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Answer/comment:

It is hard to imagine that viruses that infect as distantly a related organism as shrimp could harm the health of humans. However, some shrimp viruses come from groups of viruses that include strains which infect humans (Timrnoney et al., 1992), so it may be incorrect to entirely rule out any possibility of human health effects under any circumstances.

IHHN (Parvovirus): There is no evidence that humans can be infected by Parvovirus strains that naturally infect other animals (e.g. Feline Panleukopenia, Canine Parvovirosis, Bovine and Porcine Parvovirosis, Aleutian Disease in Mink are not transmissible to humans) (Timmoney et al., 1992).

TSV (Picornavirus). There is no evidence that Picornavirus strains affecting other animals can be zoonotic (transmitted from animals to humans). However, there are two reports of humans becoming accidentally infected when manipulating vaccines (Timmoney et al., 1992).

YHV: (probably a Rhabdovirus, (Lightner 1996b)). Two diseases caused by Rhabdovirus are zoonotic, Rabies and Vesicular Stomatitis. Vesicular Stomatitis virus also infects arthropods and plants. Transmission to humans by ingestion of affected animals has not been demonstrated (Timmoney et al. 1992).

WSBV: To the best of my knowledge, baculoviruses do not infect vertebrates.

Questions:

9. U.S. aquiculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquiculture operations as a source for the virus?

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

Answer/comment:

The scanty information currently available concerning viral infections of wild shrimp populations is completely inadequate to indicate the source of infection. Both aquiculture facilties and processing plants could be sources.

Evidence from the shrimp fishery demonstrates that farmed shrimp escape aquiculture facilities, potentially spreading disease. For example, in fall 1997 shrimp fishers harvested nonnative *P. vannamei* – almost certainly of farmed origin -- in Matagorda Bay, Texas. However, this evidence in no way negates the possibility that shrimp processing plants could also be a source of shrimp viruses.

On a related topic, disease outbreaks on US shrimp farms suggest that disease eradication on shrimp farms should be a vital element of efforts to prevent the establishment of shrimp viruses in wild populations of shrimp and other susceptible organisms. Although the JSA reports states that there are no reliable procedures for pond disinfection (p. 25), there are well-regarded procedures for cleaning up an aquiculture facility that has suffered a disease outbreak (Bell and Lightner 1992).

Question:

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquiculture and wild populations, with regard to shrimp viruses?

Answer/comment:

Just because it appears unusual for domesticated animals to infect wild animals with disease does not mean that such disease transfers cannot have severe consequences and that the potential for disease transfers should not be of considerable concern. Consider the following relevant evidence:

● Aquiculture can be a source of new pathogens, parasites, and other organisms harmful to wild populations. The Japanese oyster drill (*Ocenebra japonica*) and a predatory flatworm *(Pseudosylochus ostreophagus)* were introduced with the Pacific oyster and have contributed to the decline of west coast oyster stocks (Clugston 1990).

● At least some experts consider the spread of exotic pathogens to wild fish to be the greatest threat to wild fish from salmon netpen farming (Kent 1994). Escaped farmed salmon may have been the source of the disease furunculosis in Norway, which has killed large numbers of wild fish (Heggberget et al. 1993). However, the evidence that freed salmon have spread new diseases to wild salmon is not "airtight" (B.C. Environmental Assessment Office, 1997).

● The devastating spread of Asian chestnut blight to American Chestnut trees clearly demonstrates that introduced diseases can nearly eradicate a species (albeit a terrestrial plant species), radically change an ecosystem, and cause economic harm. American Chestnuts once dominated Appalachian forests (Keever 1953). Chestnuts were nearly eradicated following the inadvertent introduction early in this century of Asian chestnut blight on nursery stock of Asian chestnuts. Because of the introduction of this ascomycete-pathogen, Appalachian forests are now dominated by an oak-hickory complex instead of chestnuts (Keever 1953), and some researchers believe that ecosystem function (i.e. rates of nutrient cycling) may have changed in these forests (Shugart and West 1977). Moreover, the logging industry once supported by chestnuts – tall, straight hardwoods -- was ended by Chestnut blight.

Question:

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Retailers and consumers should receive additional evaluation. Retailers and consumers may wash shrimp, using water that flows to municipal sewage and individual septic systems that may not deactivate viruses. Similarly, feces from consumers than have eaten uncooked shrimp (e.g., in ceviche) could contain active viruses that are not deactivated by sewage treatment.

Question:

13. After considering the sources addressed in the shrimp virus report, what sources other than aquiculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

Answer/comment:

Given time constraints, bait shrimp are the most critical source for evaluation after aquaculture and shrimp processing. Shrimp are a popular form of bait in the southeastern Atlantic and Gulf of Mexico. Bait shrimp are often imported and are “released” directly into coastal waters.

Question:

14. Is manufactured shrimp feed a potential virus sources, or is the processing temperature sufficient to rule this source out?

Answer/comment:

The high temperatures at which shrimp and other animal feeds are typically processed are likely to greatly reduce, if not eliminate, the risk of transmission of shrimp viruses in feed. However, to the best of my knowledge there are not data to substantiate this assertion for all the shrimp viruses considered in the JSA report.

To make shrimp meal for feeds, shrimp byproducts are cooked in an oven at 90-95 C and then dried (Autin 1997). Feed manufacturing companies then process shrimp meal under different temperature-time regimes, depending on the final product being made. According to one US feed manufacturer, 99.9% of shrimp feeds manufactured in the United States are processed at temperatures of 76.6- 137.7 C, with most feeds subjected to 87.7-110 C (T. Ziegler, Minutes of the stakeholder meetings) – although he does not mention the length of time that high temperatures are maintained.

Flegel (1 995) reports that YHV was inactivated by exposure to 60 C for 15 minutes, concluding that YHV is not transmitted by shrimp feeds. IHHNV is inactivated at 80 C (Al-Mazrooei 1995, cited in Lotz 1997). There appear to be no data concerning time-temperature inactivation of TSV and WSSV. However, potentially relevant to WSSV, another shrimp baculovirus, *Baculovirus pennaei,* is inactivated in 10 minutes at temperatures of 60-90 C (LeBlanc and Overstreet 1991, cited in Lotz 1997).

In short, US shrimp feeds are unlikely to transmit YHV or IHHNV. Data about temperature-time regimes to inactivate TSV and WSSV are clearly needed. Compared to many of the other data needed to assess the risks of shrimp viruses, collection of data concerning inactivation of TSV and WSSV should be relatively quick and straightforward – and should be a high priority.

Question:

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Answer/question:

Shrimp populations fluctuate considerably from year to year – a 25% change is not uncommon in the Gulf of Mexico. Shrimp population models based on physical factors such as temperature and on recruitment strength and used to forecast shrimp harvests have historically been fairly accurate in predicting population fluctuations (J. Nance, pers. comm to P. Baker).

A large disparity between the harvest predicted by a forecasting model and an actual shrimp harvest - as there was this past season in the western Gulf of Mexico – could indicate shrimp mortality from a virus. However, viral disease would be only one of a number of possible explanations for an unexpected reduction in shrimp harvests.

Low levels of mortality from shrimp viruses would likely not be detected by comparing the results of predicted and actual shrimp harvests.

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Howard Harder

PRE—MEETINGCOMMENTS

Management goals, assessment endpoints and the conceptual model

1. The management goal is adequate for what is going to be the initial phase of a ongoing investigation.

2. The stated assessment endpoints are sufficient. Shrimp viruses presumably will affect the target animal to a greater extent than they will affect related organisms. From the evidence to-date, determining whether or not shrimp viruses have an ecologically significant effect on wild shrimp populations will be chanllenge enough for the workgroup.

3. This is overly ambitious for the initial phase.

Viral stressors and factors regulating shrimp populations

4. In terms of the magement goal, there is both relevant and irrelevantlaboratory information on the infectivity of viral agents to our native shrimp species. Much of the public's concern originates from reports that native speices are affected by various viruses. The vast majority of these reports comes from studies that cause infection by direct syringe injectionof the viral agents and, therefore, the public's reacfion is based on non-applicable information. As risk assessment is based on probabilities, it seems reasonable to base decision making on the most probable vectors of per os or water borne exposure, not the least probable vector of transmission through hypodermic use. (Even per os studies may not mimic the real worl if the fed material is heavily loaded with viral particles and/or if infected material is the sole food source for the test animals, but these types of studies currently allow the best assessment of actual risk.)

5. Inoculations seem to work for a wide range of animals; it could be assumed that they would be effective for shrimp. The wild population of *Penaeus vannamei* in Central America apparently has been inoculated with Taura Syndrome Virus (TSV) and the only lasting effect on the population level seems to be an increased resistance to the disease. A much less studied situation in South Carolina suggests the same conclusion as the wild population of *Penaeus* setiferus contains a "White Spot Virus" that has been in the population for a number of years with no noticeable affect on population numbers (see question 10). (This virus will be labelled WSV in this paper to distinguish it from the Asian White Spot Syndrome Virus (WSSV)). This WSV only expressed itself when the shrimp were confirmed and stressed. This virus has now been found in shrimp from Georgia, and (anecdotally) from Texas and Washington state.

6. With the exception of catastrophic viral outbreaks (die-offs) in a wild population, it may be impossible to separate natural or man-made effects on population levels from subtle effects of diseases. For example, in South Carolina, it has been documented that winter temperatures are a major factor in determining the magnitude of the following fall harvest of white shrimp which can vary by a factor of 2; a disease outbreak with a mortality rate of 20 to 30 percent may not be detectable.

7. With no evidence to the contrary, it can be assumed that there is no effect on humans by the viruses Of concern in this report. With the millions of pounds of *virus-laden shrimp* that is imported *and consumed* yearly with reports of health related *problems,* even among individuals with depressed immune system this seems reasonable certain. This conclusion should be bolstered by the amount of shrimp eaten raw as sashimi.

8. This should be considered a two part question asking botb if the current identification techniques adequately reliable, and are there enough identification centers available. The first part is better left to the disease experts, but the second question is easily answered shrimp aquaculturists. There are enough facilities and experts to allow for all phases of disease screening that is desireable for the culture industry in the United States. With so few centers available and the volume of ''routine" analyses (from both within aud without the U.S.) they are asked to perform it is inevitable that backlogs develop. Rapid identification AND confirmation of diseases is all-important, yet is currently not possible as even with priority given to samples from outbreaks, definitive results can take weeks. Culture facilities face a two-edged sword as it is deasirable to hold animals until their disease free status is assured, yet the longer they are held at high densities, the more stressed they become and the more suseptable they are to infections, both from outside vectors and from forcing the expression of latant diseases.

Viral pathways and sources

Aquaculture

9& 10. Aquaculture operations can be a source of viral introduction but existing evidence indicates that the introduction is confined to the culture facility. With the exception of the Gulf of California study discussed elsewhere, are there any instances where outbreaks originated on a facility and significant mortality subsequently occured outside the facilty?

Harder

In South Carolina, three viruses have been identified over a number of years. In the late 1980's and early l990's, IHHNV was a problem on several farms. It was known that this virus was imported with *P. vannamei* post-larvae *(pl's)* but was considered a risk associated with the culture of this species. During these years there was no restriction on water discharge and there were unitentional releases of infected animals from the farms. Over the last 10 years, no native shrimp have been diagnosed with IHHNV.

ln 1996, a number of farms experienced a massive TSV outbreak. Again there was water exchange prior to recognition of the disease and there was significant bird predation on dead and dying animals, with likely transport of tissue and feaces to the surrounding environment. Despite intensive monitoring of the wild population subsquent to this outbreak no noticeable effects were observed; indeed, the following year was a "bumper crop" of native shrimp.

WSV was discovered in South Carolina during the winter of 1996-97. Two separate and discreet collections of adult *P. senferus* (to *be* overwintered as broodstock) were taken to a state agency and a private farm, respectively. Despite the two collected populations being captured, transported, and held separately, both groups exhibited low-grade, but significant, mortalities that was diagnosed as some form of a White Spot Virus. These circumstances indicated the virus was present in the wild and a subsequent survey of areas along the South Atlantic coast confirmed the presence of the virus in shrimp and other crustaceans. The historical presence of the disease was confirmed in archival samples from previous years. In 1997, at least one farm experienced an outbreak of this WSV in ponds stocked with *Pemaeus stylirastis* (that had previously tested negative for WSV), illustrating that the movement of the disease was from the wild to the farm.

Shrimp processmg

11. & 12. It is a certainty that processing plants have processed virus-infected product for years, and retailers have sold virus-infected product for years without question, discharges from plants that processed infected shrimp have reached the environment, and retailers have sold infected shrimp tthat ended up as bait. Whether this processing or selling constitutes a significant threat to wild populations is unknown.

Shrimp processing

13. **The WSV in South Carolina apparently was not introduced by aquaculture as it was never** identified in any of the farmed species. If aquaculture is ruledout as an introducing vector,

then this virus was introduced by another vector or was a naturally occuring disease in the native population. This would seem to argue for the tiered approach with the first assessment being to determine the naturally occuring diseases in the areas and species of interest.

14. Discount manufactured feed as potential vector. Shrimp farms should be discouraged from using or supplementing with, natural feeds such as baitfish, trawler by-catch, etc.

Stressor effects

15. In the best possible light, there is no evidence of effects of introduced viruses on wild shrimp populations. In addition to a possible viral cause, there seem to be indications that non-viral causative agents may be responsible for the temporary decline in the wild populations of shrimp in the Gulf of California in the 1980's attributed to the upper reaches of the Gulf. (Mexican officals have said that a combination of weather conditions and overfishing may be determining factors in the population decline.) Shrimp population numbers are characterized by cyclical fluctuations over time in the absence of viruses, and it is questionable as to whether potential viral impacts on these numbers can be separated from all of the other impacts. In the worst light, IHHNV caused a reduced harvest of shrimp in the Gulf of California for several years before the population rebounded.

The TSV situation in South America underscores the importance of addressing the concerns presented in question 16. TSV was identified and largely confirmed as the causative agent in massivse pond mortalities in cultured shrimp. Subsequently, TSV was identified in wild shrimp from surrounding areas. Without background data, is it not possible, even likely, that TSV was endemic to the wild population and unnoticed until it entered the culture environment and amplified? This would be similar to what is suspected with the WSV that appears to have been endemic in South Carolina for some time.

16. It is critical that background information be available for any type of risk assessment. In some cases it is possible to track a viral outbreak to a particular source, such as a farm experiencing a disease event and the problem being traced back, through infected pl's, to a hatchery. In other cases it is not known where the diesease originated and without background ingormation, the possibility of a culture pond being infected from the wild cannot be discounted.

17. Given the current lack of scientific information concerning all aspects of viruses in the wild, and the natural fluctuations in populations over time, it appears unlikely that trends in

population numbers, or population models, can be of significant use in interpreting effects of viruses on wild populations. (See questions 15 & 16)

18. Perhaps if a conclusion is reached that viruses do pose significant risks to native shrimp poplulations, then further investigations of risks to other organisms are warranted, but not at this time. (See question 2)

Comprehensive risk assessment and research needs

19. One significant contribution of the risk assessment approach should be to present a concise and factual report of what is, and is not, known about the shrimp virus situation. It appears that interest groups only present information favorable to their point of view and the media only reports items that may appear sensational; which often leaves the general public mislead.

Similarly, this review and workshop should allow all participants to be more fully informed of the current state of viral affairs; experts in one field rarely get the opportunity to see the "big picture" in "real time".

20. Some immediate needs: Background assessment of current state of the wild population with respect to the incidence of viral occurance; complilation and dissemination of available pertinent literature on viruses and their effects. Unsure of timeframe and cost.

21. & 22. Should be considered after workshop.

Fritz Jaenike

WRITTEN COMMENTS FOR “CHARGE TO PANEL MEMBERS”

Fritz Jaenike

1. The management goal reflects the dimensions of the shrimp virus problem, but needs to be qualified a great deal when utilizing it & the comer stone of the whole process. Some of the qualifiers which should be considered include:

   A. What is a “new” virus versus an “established” one? How do we know that background levels of virus are not naturally occurring or already present? i. e. /in South Carolina WSV (or WSV like) is widespread and detectable in a number of marine and estuarine species. Is this considered a “new” virus?

   B. When considering “disease-causing” viruses can you accurately lump all viruses into the same category or not? The disease causing abilities of IHHNV is certainly much different than WSV with regards to Gulf of Mexico and S. Atlantic shrimp species, which have been tested in the laboratories. Should imported shrimp with WSV be considered differently than imported shrimp’ with IHHNV) In aquiculture we would consider them quite differently when evaluating risks.

2. The first assessment endpoint in the jSA report “ Survival, growth and reproduction of wild Penaeid shrimp populations in the Gulf of Mexico and southeastern U. S. Atlantic coastal waters.” is already so broad that it will be hard to measure. To expand the endpoint further to the entire marine ecosystem seems completely burdensome. The second endpoint of ‘Ecological structure and function of coastal and near-shore marine communities as they affect wild Penaeid shrimp populations.” is a Multiyear undertaking that will probably lead the assessment to remain unresolved for years. If the risk assessment

is tiered and the policy makers decide “better safe than sorry” until we know the answers to all the questions, then we may end up with unrealistic recommendations and fail to determine a clear, realistic course of action.
The determination of more specific answers related to virus policies may not be accomplished with goals that are so broad. Of course I would like to have an additional assessment endpoint. Protection of Shrimp mariculture industry from imported shrimp viruses.

3. A broader assessment considering alternate seafood production methods or other land usages as stressors to the health of the natural shrimp populations would be a huge undertaking. Deciding which of these stressors would be likely to antagonize or be synergistic to background viral levels in wild shrimp would be even more difficult. By broadening the assessment we may lose focus of the intended outcome of the questions at hand. The main concern seems to be focused on shrimp viruses as it relates to risks to native shrimp populations. What should be the policy of the government on the imports of viral containing shrimp or with regards to outbreaks on aquiculture fins?

4. We need to evaluate the trials conducted in Texas on tSV and native shrimp as an example. Lab trials demonstrated problems with PL *P. setiferus*, while field trials failed to show similar effects. Lab trials are too intensive to be widely utilized to predict wild population effects. It does, however seem relevant to assume that if you can’t kill shrimp in the lab it should be considered very low risk for a problem to occur in wild populations.

5. Very likely. The use of wild *P. vannamei* in ponds in Central and S. America over several years has generally demonstrated a decreased susceptibility y to SV with time. Gulf of California *P. stylirostris* utilized in aquiculture are

demonstrating less susceptibility to IHHNV than in previous years. Some strains of *P. stylirostris* have even been selected in aquaculture and are now resistant to IHHNV.

6. It's tough to say since past information on natural swings in shrimp populations are not associated with analyses which substantiate presence or absence of viruses. If you take historical information on shrimp population variations, the determination of which environmental or human activity was the major or most likely cause has seemed very subjective. A quantitative basis for determining variation is lacking.

7. From the bulk of historical information I would think the risk factor of shrimp viruses harming humans could be reduced to next to zero if not zero.

8. Some are some are not. Dr. Don Lightner could answer this question best. The need for holding in shrimp in stressful conditions followed by bioassays on known susceptible species is probably the most reliable indicator vs. some diagnostic tool by itself This would particularly be the case with environmental media.

9. Information on the wild populations is so sketchy and incomplete it's hard to base any conclusions. Texas and South Carolina facilities operated with IHHNV present in pond raised shrimp for several years. I have not yet heard of a positive IHHNV occurrence in the wild populations. South Carolina may now be the Ieading information source on virus in wild populations utilizing the newest diagnostic tools. It appeared that WSV was present in the wild population prior to its detection in any aquiculture facility. More examples on how the wild

populations are a source of virus to aquiculture exist in other countries where wild seed are used in ponds.

10. This observation is very prevalent for shrimp viruses in South America, Mexico and Asia as evidenced by information from Roland Laramore on TSV in wild *P. vannamei* which is published in the JSA report.

11. There is not a great deal of information on the viral status of local wild shrimp utilizing the most recent diagnostic tools to base any opinions on. I am not aware of any survey on the viral status of wild shrimp from areas adjacent to major processing areas located in Alabama, Mississippi or Louisiana. The most data points on viral status of local shrimp that I am aware of is in South Carolina and I have been told there is not a great deal of processing going on there.

12. Yes, retailers, restaurants and food service.

13. Importers besides processors, bait and ship ballast water. Importers.

14. Not a source according to Tim O'Keefe with Rangen Feeds.

15. With caution. The role of IHHNV in the decline of shrimp populations in the 1980's needs to be considered along with the other stressors to the populations. How can we be sure that the viruses were introduced versus being at some baseline concentration within the wild population then expressing themselves in aquiculture and or environmental stress situations?

16. There should be a database established on background levels of viruses in wild shrimp populations utilizing the most sensitive diagnostic tools. Concentration

and holding of shrimp populations may need to be done to obtain low level or baseline levels of some viruses. samples from processing areas which have not been surveyed should be prioritized in addition to aquiculture areas and control areas where neither exist. Use what information is available but rely on sensitive diagnostic techniques or those utilizing amplifications. This data gap should not be assumed for a risk assessment.

17. with or without analyses its tough to pin a decline on a virus. Shrimp population models are tough to use due to the number of factors such as weather which can cause normal variations.

18. There is need to evaluate what the case was in the Gulf of California with regards to non-shrimp species during the shrimp decline of the late 80's. The non-shrimp invertebrate populations in Asia where WSV and YHV are known occur and to be carried by other invertebrates besides shrimp should be evaluated.

19. Information from a risk assessment can contribute much to management decisions. South Carolina as a case point which is presently occurring should be considered. A lot of data should be evaluated in terms of effects on wild populations to help in determining management decisions. If we can identify the most likely problem causing viruses and the areas in which they are handled we can manage accordingly.

20. Gather more information in South Carolina. I don't know how much it will cost, but a concerted effort should produce some results relatively quickly.

21. Yes, we should prioritize the most likely inputs of virus to the U. S. (imported shrimp), and decide how best to implement practical, cost effective precautionary measures.

22. First specific exposure scenarios should be identified and ranked according to most exposure to least. Then pole the stakeholders in those respective areas of measures which could be practically implemented to reduce the risk of exposure.

Donald Lightner

Lightncr

COMMENTS ON THE DOCUMENT:

MINUTES OF THE STAKEHOLDER MEETINGS OF THE JSA SHRIMP VIRUS WORK GROUP

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December 14, 1997

Management goals, assessment endpoints, and the conceptual model

1. How well does the management goal reject the dimensions of the shrimp virus problem?

On page 14 of Appendix D, Report of the "JSA Shrimp Virus Work Group" the management goal is given as:

"Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, and aquiculture operations."

In late 1995 and early 1996, when the shrimp virus issue was emerging, this goal may have been appropriate. The viruses, TSV, IHHNV, WSSV, and YHV (= Taura Syndrome Virus, Infectious Hypodermal and Hematopoietic Necrosis Virus, White Spot Syndrome Virus, and Yellow Head Virus, respectively), were "new" at that time in the sense that none of the three had been previously detected in farm raised or wild shrimp in Texas or elsewhere in North America. The management goal was based on the premise that none of these agents had become established in U.S. coastal or surface waters. There is increasing evidence that at least one of these agents, WSSV, has become established in wild stocks of the white shrimp, *Penaeus setiferus in the Gulf* of Mexico and in the western Atlantic off South Carolina. Hence, this management goal, as written, may no longer be appropriate, at least for this virus, in U.S. coastal waters.

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or alternativeIy, to the aquiculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment

Two "endpoints" are given in section 3, page 18 of the JSA report. The first centers around assessment of the threat of the shrimp viruses to *"survival, growth, and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters"*, and the second on assessing the effect of the viruses on the *"ecoIogical structure and function of*

coastal and near-shore marine communities as they affect wild penaeid shrimp populations. "

At first glance these *seem* to be reasonable "endpoints" of the risk assessment. The first is far more straight forward than the second. Some aspects of the first endpoint can be tested in controlled laboratory studies. Nonetheless, as pointed out by the JSA document and by various other persons in the "Proceedings of the Stakeholder Meetings", there are numerous important data gaps for all four shrimp viruses that will need to be filled before the JSA (or other group) can make an informed assessment on these "endpoints." Although the studies required to fill these data gaps are desirable, the time and resources required to run even a portion of the required studies is substantial if not impossible. For example, how can a study be run to determine the effect of an introduced pathogen on an ecosystem without actually introducing the pathogen? Hence, I have to recommend that the "endpoints" be kept narrowly focused (as the JSA report has generally attempted to do) so that meaningful data can be generated and used in the risk assessment process.

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

It is not at all clear to me what is being suggested here. Is it being suggested that all anthropogenic changes (i.e. alternative land uses) to coastal areas be considered in the shrimp virus risk assessment? Hence, without having the suggestion (or question) clarified I cannot comment.

Viral stressors and factors regulating shrimp populations

4. ***How relevant to virus effects on wild shrimp populations is information on infectivity and effects that is derived from laboratory or intensive aquiculture operations?***

Data of the sort referred to here, which is obtained from laboratory studies or from intensive aquaculture operations, provides an indication of the potential effects of a given "stressor" or "factor" on wild shrimp populations. Correctly run laboratory studies test only one variable. The environmental conditions in aquaculture farms is highly controlled and thus the number of variables,

while more than in a lab settings, is far less than in a "wild setting". Hence, while such data provides only an indication of what might be, it is the best and most reliable data available.

5. ***How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival overtime?***

The available data on this question suggests that it is very likely that wild shrimp populations will develop "resistance" (the term "immunity" may bean inappropriate term in arthropods) to introduced viral pathogens. Penaeid shrimp have an extremely high fecundity. This high fecundity, paired with natural selection for resistance to a given pathogen (in the continuous presence of the pathogen), translates into a high potential for the relatively rapid development of specific pathogen resistance with each successive generation. Only survivors that are resistant to a particular pathogen live to breed. This phenomenon has occurred in the wild *P. stylirostris* stocks in the Gulf of California in response to the introduction and establishment of IHHNV. It has been used in the development of specific pathogen resistant (SPR) breeding lines (for IHHNV and TSV) by several groups in the shrimp farming industry. Perhaps, the apparently steadily improving resistance of wild postlarvae used in Latin American shrimp farms to TSV has likewise resulted from natural selection of some wild stocks of *P. vannamei* where the virus has become enzootic.

6. ***How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?***

To obtain the sort of information required here, single (or multiple factors) have to first be identified and defined. Then controlled laboratoty studies, in which the effects of varying the values of single (or multiple) factors, can be designed and run to gain some insight as to their potential effect in natural settings. When coupled with controlled virus challenge studies, the effect of some factors such as changing salinity, temperatures, or other natural or non-viral factors, can be estimated.

Z Can human health effects from shrimp viruses be ruled out as a concern ? Why or why not?

Nothing in living systems is absolute. However, shrimp viruses can only affect human health indirectly through loss of income: shrimp that die from virus infections cannot be harvested (from farms or the wild) and sold. Despite the opportunity for infection presented over the past 30 to 50 years by the millions of tons of shrimp that have been harvested from all over the world from wild fisheries and farms, have been processed packed and cooked by human hands, and finally consumed by humans, no case of a shrimp virus infecting a human (or any other mammal) has ever been reported.

8. ***Are the available identification techniques for shrimp viruses reliable enough to allow definite conclusions to be drawn about the occurrence of viruses in shrimp and environmental media ?***

This question can best be addressed with the following table. The table lists most of the methods available for the detection of infections by the viruses TSV, IHHNV, WSSV and YHV. Good methods for detection of infection are readily available for all but YHV. Application of these methods to "environmental media" maybe more problematic, and is largely untested.

Table 4. Summary of diagnostic and detection methods for the major viruses of concern to the shrimp culture industries of the Americas (modified from Lightner 1996a).

Method*	IHHNV	TSV	YHV	WSSV
Direct bright field light microscopy (LM)	-	++	++	++
Phase Contrast LM	-	-	-	+
Dark-field LM	.	-	.	++
Histopathology (of acute infections)	++	+++	+++	++
Enhancement/Histology	++	+	-	++
Bioassay/Histology	++	+++	+++	++
Transmission electron microscopy (EM)	+	+	+	+
Scanning EM	.	+	-	+
Fluorescent antibody with PABs or MAbs	r&d	r&d	r&d	r&d
ELISA with PABs		r&d	r&d	
ELISA with MABs	+/r&d	++/kit	r&d	
DNA Probes	+++/K	+++/K	+/r&d	+++/K
PCR	+++	++/r&d	+/r&d	+++

** Definitions:

- = no known or published application of technique.
+ = application of technique known or published.
++ = application of technique considered by author to provide sufficient diagnostic accuracy or pathogen detection sensitivity for some applications.
+++ = technique provides a high degree of sensitivity in pathogen detection.
K = diagnostic kit or product available from DiagXotics, Inc. (Wilton, CT, U.S.A.).

Methods: BF = bright field LM of tissue impression smears, wet-mounts, stained whole mounts;
LM = light microscopy,
EM= electron microscopy of sections or of purified or semi-purified virus;
ELISA = enzyme-linked immunosorbent assay;
PAbs = polyclonal antibodies;
MAbs = monoclinal antibodies;
r&d = techniques in research and development phase.

Viral pathways and sources

Aquiculture

9. U.S. aquiculture operations have had problems with viral diseases for several years. How does informtation from local wild shrimp populations support or refute the importance of aquiculture operations as a source for the virus?

While shrimp farms in the United States have had a history of disease episodes caused by IHHNV, TSV, WSSV, and possibly YHV, only strains of WSSV have been detected in populations of wild shrimp. Because only specific pathogen-free (SPF; shown to be free of IHHNV, TSV, WSSV, YHV, and other major shrimp pathogens by routine testing over multiple generations in captivity) *P. vannamei* or indigenous *P. setiferus* had been cultured at the affected farm in 1995 (and in 1993 and 1994), the probability is extremely low that the *P. vannamei* stocks were the source of TSV, WSSV, and YHV that appeared in Texas in 1995. Contamination of the affected farm (TSV in May, and WSSV and YHV in October, 1995) came from some other source. Likewise, monitoring of the stocks used at the farms in Texas and South Carolina in 1996 and 1997, clearly demonstrated that TSV entered some farms that year through a breech in the SPF program. However, WSSV and the YHV agent were not detected in the *P. vannamei* stocks used in 1995-1997, unless wild *P. setiferus* was also present. These data implicate shrimp farming only in there-occurrence of TSV the U.S. in 1996, but not in initial appearance of TSV in Texas in 1995, nor of the appearance of WSSV and YHV in 1995 and 1997. Wild *P. setiferus* have been clearly shown to be the source of contamination in these latter cases.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquiculture and wild shrimp populations, with regard to shrimp viruses?

First of all, the basic premise of this question is wrong! It is not difficult to find examples in the literature (in mammals, birds, fish, mollusks, and crayfish) where serious pathogens (viral, bacterial,

protozoan, and fungal) have been transferred from domesticated (or captive non-indigenous) stocks to wild stocks. Reducing the risk of accidental introduction of non-indigenous pathogens to wild stocks with introduced domesticated or captive-wild stocks are among the expressed purposes of the ICES Guidelines and of the USMSFC SPF program.

Shrimp processing

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does informtation from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus ?

The answer depends on the virus. While apparently not enzootic in the U. S., IHHNV and TSV are enzootic in cultured and wild shrimp stocks in most shrimp farming areas of North America. WSSV and YHV are not. Other than in Asia and the Indo-Pacific, WSSV and YHV have only been found in wild or cultured shrimp in the US. If we look at what is different between the U.S. and other major penaeid shrimp farming or fishing countries in the Americas, it is apparent that one difference is that the U.S. imports and processes vast quantities of Asian shrimp, while the other countries, who have not yet had cases of WSSV or YHV, do not import and/or process shrimp from areas where WSSV and YHV are prevalent.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Yes. Sport fishermen commonly purchase penaeid shrimp from retail outlets (grocery stores, as well as from specialized bait dealers) and introduce these potentially contaminated shrimp where they fish. Imported shrimp are commonly used as bait in marine, estuarine, and freshwater sport fisheries in the U.S.

Other potential sources and pathways

13. After considering the sources addressed in the shrimp virus report, what sources other than aquiculture and shrimp processing are most Critical for evaluation in a risk assessment of shrimp viruses ? Given time constraints, which of these should be the focus of discussion at the workshop?

Bait shrimp should be considered. Ship ballast water, visitors, birds, feeds and feed ingredients, and other vehicles of transport are far less likely to provide an effective means of virus transport than are the live or frozen hosts of these pathogens. Therefore, all live and frozen shrimp products should be the focus of discussion at the workshop.

14. Is manufactured shrimp feed a potential virus source or is the processing temperature sufficient to rule this source out?

As I answered to one of the questions earlier in this discussion nothing is absolute. However, the relative risk posed by shrimp feed (that contains shrimp or crab meals) is extremely low. Were this not the case and shrimp feeds were the source of these viruses in the U. S., other countries using far more shrimp feed from the same sources, should have been even more severely impacted by the pathogens in question than has the U.S. industry.

Stressor effects

15. ***How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted?***

Volumes could be written on this question. The effect of an introduced virus on a wild population is affected by several factors. Among the most important of these are: 1) the relative naivety (susceptibility) of the host population to the virus; 2) the virus' mode(s) of transmission; 3) efficiency of transmission by horizontal or vertical routes; 4) life stages when acute disease typically occurs; 5) environmental factors that could influence disease expression at the susceptible life history stages; and 6) other factors. With this in mind, the available evidence should be considered

individuality for each virus in each host system. For example, the prognosis for an IHHNV infection in naive *P. stylirostris"* in the Gulf of California in 1988-1992 is not the same as the prognosis for TSV infection in wild Ecuadorian *P. vannamei*. We know from controlled laboratory studies that the latter resulted in more survivors than the did former.

16. There is presently a lack of basic data on background levels of pathogenic viruses in wild shrimp populations in U.S. waters. How should this "gap be evaluated in a risk assessment?

There have been a number of pathogen and parasite surveys carried out on shrimp from U.S. waters. Some of these date back to the 1960's; some have been thorough multi-year studies in which samples of shrimp in various life stages were taken and examined for viral or other pathogens. Likewise, the academic and commercial aquaculture industries in the U.S. have collected, cultured and studied wild shrimp on and off since the late 1960's. From all of these studies, BP is the only viral pathogen documented in wild shrimp in U.S. waters prior to 1995. While not explicitly tested for, signs of infection by WSSV, YHV, IHHNV, and TSV were not noted in these studies. Had pathogens like WSSV been present before 1995, it would have made its presence known especially in captive live animals in laboratories or bait camps. The "gap" in the data is not as large as the question implies.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Population models are only as good as the data fed into them. In order to have any validity, studies done on shrimp viruses in wild populations will require that the populations of interest are appropriately sampled and tested for the pathogens of concern. The resulting incidence and prevalence data can then be used to make predictions and draw conclusions from population models.

18. How important are potential viral effects on non-shrimp species?

This question may only apply to WSSV. For IHHNV, TSV, and probably YHV, penaeids (or very closely related shrimps) seem to be susceptible to infection and prone to disease if infected. In marked contrast, WSSV can infect, and kill in some cases, a wide variety of crustaceans. Among the hosts killed by WSSV are some species of freshwater crayfish. The wide host range of WSSV

makes it an important potential pathogen of North American crustaceans, both freshwater and marine.

Comprehensive risk assessment and research needs

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, ie., will it add significantly to the infornation presently available?

A comprehensive risk assessment has the potential of gathering virtually all of the available information on this topic in one place and extracting from it the facts necessary to make informed management decisions. The key to the appropriateness of the decisions made, may depend in large part, on how well the available data is acquired and evaluated.

20. What ***type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp population models), and what would be the likely time frame and cost?***

This question might best be deferred to the NMFS where I presume the latest models are available.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios ?

What treatment options?

22. ***Summarize the critical research needs for completing such a risk assessment?***

I cannot comment here because it is not at all clear to me what is being asked in question #2 1.

Jeffrey Lotz

Gulf Coast Research Laboratory

MEMORANDUM

From: J. M. Lotz
Date: 18 December 1997
To: EPA/ERG Shrimp Virus Peer Review and Workshop
Subject: Comments on questions

Management Goals, Assessment Endpoints, and the Conceptual Model

1. *How well does the management goal reflect the dimensions of the shrimp virus problem?*

> *"Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, and aquaculture operations."*

The genesis of this workshop appears to be the possible introduction and establishment of one of four viral agents of shrimp aquiculture in the U.S. Gulf of Mexico and the Atlantic Ocean. The agents are WSV, TV, YHV, and IHHNV. However, as is perhaps common to these kinds of activities the management goal appears to lack precision.

(a) The viruses are not specifically identified. The phrase "new disease-causing viruses" implies management of as yet unknown and undiscovered viruses. If this breadth is to be applied to viruses generally why not include other categories of pathogens and potential pathogens?

(b) What is meant by establishment? Would the finding of a positive animal in a wild populations meet the report's definition of establishment, should it be found over some period of time, should it be a self maintaining population of virus.

(c) The word "shrimp" implies more than *P. aztecus*, *P. duorarum*, and *P. setiferus*.

Jeffrey M. Lotz Ph.D.

(d) The word disease-causing is a very slippery word. If infected animals are not seen to be diseased are they not to be considered for management or does disease causing imply "potentially an agent of disease". In this case any parasite could be a pathogen in some species of host.

The second concern is that if one or more of the agents under consideration have already been introduced then the management goal can not be met and the exercise seems irrelevant to the management goal. There is some evidence that at least one of the viruses have already been established in both bodies of water.

The goal as stated ranks the endpoints. Highest priority is prevention of establishment of the viruses. Taking second position is the minimization of impact on business. Otherwise the wording would be something like "minimize the probability" or "reduce the probability" of establishment. If the goal is to guarantee that new viruses are not established (the phrase says "to prevent" not "to reduce the chance s") from aquiculture then there can be no aquiculture if the goal is to guarantee no establishment from imported shrimp then there can be no imported shrimp. My guess is that the goal is really to balance the risks of establishment with the risks of guaranteeing that establishment will not occur.

2. *Comment on the scope of the risk assessment to be limited to effects of viral establishment on populations of "shrimp".*

Shrimp is in fact a rather wide category and the risk assessment is broader than our knowledge base. Broadening the assessment more will put a greater distance between our knowledge and the decisions. Nonetheless the unforseen consequences are usually the ones that come back to haunt any decision. Although I think that the overall assessment should be clearly placed in the context of the ecosystem, the effect on the ecosystem can not be the focus of the risk assessment. This is way beyond our ability to estimate.

3. *Comment on increasing-the scope to include not only viral stressors that might affect shrimp populations but also other kinds of stressors that might affect shrimp populations.*

The farther afield from the problem at hand the process gets the less valuable the process will be. Although I can understand why the risk assessment should consider the effects of viral establishment on the ecosystem, I can't fathom why this risk assessment should be expanded to include the effect of global warming or alternative land uses on shrimp populations.

Viral Stressors and Factors Regulating Shrimp Populations

4. *How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquiculture operations?*

In general information derived from laboratory studies is quite relevant to natural settings. However, one has to look at the conditions in the particular laboratory experiment or the aquiculture setting. It is often assumed that laboratory or aquacultured animals are at much higher densities than natural populations but that is not always the case. If one assumes that the Gulf of Mexico is a large aquiculture pond or a large aquarium then the conclusions based on experiments will not translate to the Gulf of Mexico. However, if one views the Gulf of Mexico as composed of a large number of aquaculture ponds or aquaria, then the results of laboratory experiments are likely to translate more realistically. If wild animals get the same dose and have access to consumption of dead animals as they do when they are taken into the laboratory or into an aquiculture setting then they will act in the wild like they do in the artificial settings.

The adjective "intensive" changes the flavor of the question? Does the question assume that the relevance of information derived from semi-intensive or extensive aquiculture is unassailable?

5. *How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?*

Assuming all else is equal and that some members of the shrimp population possess genes that would impart resistance then it is quite likely that over several generations there would be changes in the genetic composition of both the shrimp population and the viral population that might reduce

the effects of the pathogen on the dynamics of the shrimp populations. However, the ability to predict such changes assumes that the genetic traits that code for resistance to a virus are not linked to some other fitness lowering traits such as ability to avoid predators. It is often assumed that less virulent viruses are more fit than the virulent viruses but that is not always the case. If more than one pathogen was established and resistance to one did not provide resistance to the other but actually increased the virulence of the second then no net change would be observed; some members of the shrimp population would be resistant to one pathogen and not the other. If the virus was actually maintained in one species that acted as an unaffected "carrier" the resistant carrier might actually use the virus to displace less resistance species. The virulence of the virus might be unaffected by this situation. This is the case with crayfish plague in Europe where introduce resistant crayfish are displacing wild susceptible crayfish by carrying crayfish plague.

6. *How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?*

It is always difficult if not impossible to separate the effect of two factors that operate at the same time particularly if they co-vary. What is needed is a series of natural experiments, that is, several populations of host, some with the virus some without the virus, some subject to the anthropogenic stressor some not, and some with combinations of the various factors. The populations can be separated by either time or space. In time one could look at a population prior to the introduction of a virus but with the second factor present then compare the population after the establishment of the virus. Occasionally one can use data from an unrelated host and parasite that mimics the situation of interest to determine what might in an analogous situation.

7. *Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?*

I am not concerned with the human health effects of shrimp viruses. However, one can never be absolutely certain that a virus of a non-human host will not become infectious to humans. Influenza viruses jump from pigs, chickens, etc. to humans regularly. In addition viruses of insects are transmitted to humans all of the time. The arboviruses multiply in both human and arthropod hosts. Nearly anything is possible.

8. *Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?*

In general yes; however some are more reliable than others. The question should not be asked outside of an understanding that the reliability of any single diagnostic test can only be determined after lengthy evaluation and clinical trials. Clinical trials have not occurred for the shrimp diagnostic procedures to the extent that they have for pathogens of poultry or cattle or humans. Further the trials that have been done have not been done for surveys of wild shrimp. For the most part the viruses are new, the diagnostic procedures are new, and even the aquiculture of shrimp is new. Most of the molecular diagnostic tools have not been adequately tested to be used on wild shrimp without a second backup benchmark. The typical benchmark diagnostic test is a histological exam; however, in critical cases, particularly for surveys of wild shrimp, follow-up bioassays are required. In some cases the histological evaluation is not completely reliable. The histological pathology associated with some of the viruses may look like pathology caused by another virus.

Viral Pathways and Sources

9. *U.S. aquiculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquiculture operations as a source for the virus? .*

In the U.S. there is evidence that a shrimp virus maybe present in in wild populations but the source is not known. There have been small surveys of penaeid shrimp in the U.S. for evidence of the viruses but those surveys have not turned up conclusive evidence that any of the four viruses are present in U.S. waters. The introduction of IHHNV into the Gulf of California is the best documented case of an the introduction of a viral pathogen into wild shrimp populations from aquaculture. It also may be that Taura virus has been introduced into wild shrimp in parts of Central and South America and that introduction was from aquiculture. The difference between the likelihood of aquiculture as a source for the introduction of viruses into Mexican, Central and South American wild shrimp probably lies in the much higher aquiculture levels that occur in those regions.

10. *It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquiculture and wild shrimp population, in regard to shrimp viruses?*

The situation in terrestrial livestock agriculture may appear to be different because of differences between the states of development of terrestrial agriculture and aquiculture. The vast majority of livestock used in terrestrial agriculture have no wild stocks of the same species that are exploited for commercial purposes, therefore few are concerned that say an outbreak of hoof and mouth disease in cattle will spread into wild populations of cattle. There are no wild cattle.

There are a number of examples of aquaculture as the cause for an outbreak or introduction of a disease agent into wild populations, e.g., crayfish plague, whirling disease, Anguillicola sp., several salmon bacteria and viruses. The movement of pathogens into wild species has the consequence that the wild animals then become a future source of infections once farmers eliminate the pathogen from their farmed stocks by replacement of animals imported from other farmers or regions. The wild animals are then of concern to farmers and their livestock eventhough the original introduction of the pathogen into the wild population was from aquaculture. I would not be surprised if terrestrial livestock agriculture had followed a similar scenario during its early history of domestication of stocks. Therefore the “unusual” situation in aquiculture is not unusual at all it is just that aquiculture and terrestrial livestock agriculture are at simply different phases in their development.

Shrimp Processing

11. *Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?*

The information from local wild shrimp populations is very meager. However, there is evidence that at least one of the viruses is present in wild shrimp in the Gulf and the Atlantic, The source of it is unknown and by itself doesn’t suggest processing rather than some other source. There is clear evidence that infectious virus is present in at least some frozen shrimp destined for domestic processing. Another piece of evidence that might point to processing as an indirect source is that the

U.S. aquiculture industry is the only industry in the western hemisphere that has reported WSV. WSV has not been reported from aquiculture of shrimp in Mexico, Central America, nor South America. There is much less processing of shrimp imported from Asia (where WSV is common) in Mexico, Central America and South America. This may suggest that processing of shrimp from Asia resulted in contamination of U.S. aquiculture. This of course assumes that the U.S. WSV is Asian in origin.

12. *Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?*

The evaluations that have been done are minimal; however, infectious virus has been found in shrimp in supermarkets. If these shrimp are purchased and "processed" at home the disposal of the home waste could be a source of contamination. There should be further evaluation of shrimp that may carry infectious virus regardless of whether they are to be processed or not. The focus should be on the viruses, the infectiousness of the virus, and how those viruses might contact susceptible hosts.

Other Potential Sources and Pathways

13. After considering the sources addressed in the shrimp virus report, what sources other than aquiculture and shrimp processing are the most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

One source for the establishment of a virus into U.S. waters, especially into the Gulf of Mexico, might be the natural spread of virus from a point of establishment outside of U.S. waters into shrimp of U.S. waters. In particular, it might be that an establishment in the Gulf of Mexico or the Caribbean Sea might spread into the U.S. by migration and contact among susceptible species. It would be important to know whether any of the viruses of interest are already present in areas of shrimp aquiculture along the coasts of nations bordering the Gulf of Mexico.

14. *Is manufactured feed a potential virus source, or is the processing temperature sufficient to rule this source out?*

The temperature of processing of manufactured feeds depends upon the method of preparation. IHHNV would need to be heated to 80 ‘C. It may be necessary to treat Taura Virus to an even great temperature to prevent infectiousness. There are however a number of fresh uncooked feeds that are associated with shrimp aquiculture, algae, brine shrimp, squid, and blood worms among others. A live organism could conceivably carry one or more of the viruses (WSV has been shown to have a wide host range among crustaceans). Any flesh feed could act as a mechanical vector any of the viruses. This would be particularly likely if a processing plant processes shrimp and one of the fresh feeds in particular squid is likely to be processed by the same processors as shrimp since both are used as food for people.

Stressor effects

15. *How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980’s in the Gulf of California? What about TSV release from aquiculture into the wild in South America?)*

There should be no question that viruses have been introduced into wild shirmp populations from aquaculture. Since the stated management goal of the risk assessment is to "prevent the establishment" of viruses then the pertinent data is that that can happen. When the question is, "can one predict what will happen if a virus is introduced into a wild shrimp population?" one has to again look at the available data. The data from Gulf of California clearly show that IHHNV was introduced from aquaculture and that P. stylirostris were found with IHHNV disease. What is less clear is how was IHHNV introduction related to the decline in catch. The catch data that I have seen (reported in a Tucson newspaper) is that the catch was already in decline prior to the introduction of IHHNV. I am not familiar with the data for catch of shrimp in areas where TV or WSV have been introduced. There are however, examples of the introduction of pathogens into other kinds of aquatic systems. For example the outbreak of a virus in hard head catfish (Arius felis) in the Gulf of Mexico during 1996 caused a definate short term (same year) decline in the numbers of catfish that were caught in sampling gear by state agencies in Mississippi. However, there does not seem to be any

shortage of hardhead catfish in 1997. Of course hard head catfish are not an economically important species so the numbers are not well known.

From a theoretical perspective we can consider the consequences of introducing an additional risk factor (virus) into a shrimp population. For example if the survival of shrimp in the absence of the additional factor is 10/0, that is 99°/0 of them die from some other cause and a shrimp subjected to mortality from the additional factor alone has a 75°/0 chance of dying (about the mortality rate for *P. vannamei* infected with Taura Virus) then a shrimp subjected to both the additional factor (virus) and the general mortality factors has a 99.75% chance of dying. (The chances of surviving both TV and general mortality is (1-.75)*(1-.99)=0.0025 the composite mortality rate is .9975.) The net increase due to the additional factor is only 0.75°/0, that is out of 10,000 shrimp 9900 would die in the absence of the additional factor and 9975 would die in its presence. There are certainly other considerations that need to be taken into account but the general result is that the increase in the mortality rate from the addition of another mortality factor is actually quite small when the initial mortality rate is quite high.

16. *There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U, S. waters. How should this data gap be evaluated in a risk assessment?*

The data gap can only be evaluated as lacking. I guess the reason for a risk assessment is to deal with data gaps. There may be more data than one thinks. There is at least one unpublished data set on the seasonal dynamics of *Baculovirus penaei* (BP) in *P. aztecus*. BP is a fairly pathogenic virus of shrimp that is native to the Gulf of Mexico.

17. *How can changes in wild populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp populations models be used in the future?*

I think that changes in wild populations are an extremely valuable source of information. However, one needs good data on variation over several years prior to the introduction of a virus. The data need to be appropriately collected. There are real problems with landings as indicators of shrimp numbers. If fishery independent data on abundances of shrimp are available prior to an introduction and the dynamics can be followed subsequently then good conclusion can be made. Another approach is to look at natural experiments as alluded to in my comments to number 1.

Population models of shrimp are important. More important are models of shrimp and their pathogens. These models can be very helpful in identifying what rates need to be determined and what parameters need to be estimated. For example epidemiological models can be built that incorporate the population dynamics of shrimp populations and they can be used to suggest which factors are important to the establishment of a pathogen and the consequences of that establishment on shrimp populations. Not only can population dynamic models be useful but also genetic and evolutionary modes should be considered.

18. *How important are potential viral effects on non-shrimp species?*

Very important. For example, if a virus reduces the numbers of a species that serves as food for an important fishery species then there could be a reduction in the abundance of that fishery species. In addition, other species may serve as reservoirs for outbreaks in other wild or cultured species. Certainly if the goal is to prevent establishment then the role of non-shrimp species needs evaluation.

Comprehensive Risk Assessment and Research Needs

19. *How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?*

A comprehensive risk assessment should contribute to understanding and defining what the problem is and what might be done to prevent establishment. In addition the assessment will probably point out areas for future research and information that is needed to answer specific questions related to introduction if the viruses. The process seems to be rather lengthy. Pathways are now open that appear to have a considerable amount of virus already. Establishment might actually occur before the assessment is done.

20. *What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp population models), and what would be the likely timeframe and cost?*

I think that it is important to get really good estimates of how much infectious virus is coming into the U.S. and where the virus might be contacting wild populations. I think that the most important factor in determining whether a virus will be established in a susceptible wild population is how many times introduction is tried. I think that determining whether a particular virus will become established will require detailed knowledge of the doses that wild populations are actually exposed to, the distribution of shrimp in the wild, the virulence of the virus to the species of interest, and the transmission potential of the viruses in water or by contacting infected shrimp. These kinds of parameters can be put into epidemiological models that will help understand whether a virus is likely to become established at various values of dose, susceptibility and transmissibility.

21. *Should future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?*

Yes.

22. *Summarize the critical research needs for completing such a risk assessment.*

We need to know how much virus is contacting wild shrimp populations and what the infectiousness of the contacting virus is. We need information on the transmission rate within and among wild populations of the species of wild shrimp. We need evaluation of the virulence of the viruses in the species of wild shrimp of interest. It is also critical to determine what the temporal and spatial distribution of wild shrimp populations are in the Gulf and Atlantic. This kind as well as other similar kinds of information will be needed for epidemic models that will allow good guesses for the likelihood of establishment through various pathways. Another piece of information that is needed is to know whether or not the pathogens of interest have are already established in the Gulf and Atlantic.

Roy Martin

Premeeting comments are not available at this time.

Larry McKinney

Larry D. McKinney

RESPONSE TO QUESTIONS FROM - LARRY McKINNEY

MANAGEMENT GOALS. ASSESSMENT ENDPOINTS. AND THE CONCEPTUAL MODEL

1. **How well does the management goal reflect the dimensions of the shrimp virus problem?**

   The management goal: *Prevent the establishment of new* disease-causing *viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, and aquiculture operations is* on target and appropriate for a risk assessment exercise.

2. **Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquiculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.**

   The assessment endpoints as proposed seem appropriate, although the second assessment endpoint: *The ecological structure and function of coastal and near shore marine communities as they affect wild penaeid shrimp populations* - may be too broad even in the context of a risk assessment. It is my understanding that this endpoint represents the "valued ecological entity" and that Survival, *growth and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. At/antic coastal waters - is* intended to represent an attribute of that entity, in the context of risk assessment process, that are important to protect and are potentially at risk. 1 would not recommend expanding these endpoints to include additional risks.

3. **It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.**

   I do believe that the impact of additional stressors should be assessed. Some that were included in testimony were: Operational methods, especially associated with

wastewater discharges, bait production for recreational use, shrimp feed production, human waste, direct importations to retailers. Intuitively, some would seem of low probability, but I would think they need some level of consideration.

VIRAL STRESSORS AND FACTORS REGULATING SHRIMP POPULATIONS

This topic includes basic information about shrimp viruses as well as the full range of natural and anthropogenic factors that regulate shrimp populations. Questions for consideration:

4. **How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquiculture operations?**

   It is very relevant because is establishes one endpoint in assessing the probability that wild populations could be infected.

5. **How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?**

   I cannot answer that, I lack the expertise. At least one of the studies presented as testimony asserts such an effect.

6. **How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?**

   Unless the effect of the viral stressor is significant (overwhelming), I am not sure that we have adequate data to separate out natural and non-viral anthropogenic factors.

7. **Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?**

   I cannot answer that, I lack the expertise.

8. **Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?**

While I lack the direct expertise, my review available techniques indicates that they are inadequate.

VIRAL PATHWAYS AND SOURCES

The shrimp virus work group considered aquiculture and shrimp processing to be the primary pathways of concern leading to exposure to pathogenic shrimp viruses, but is also identified a number of other potential pathways. Some related questions are listed below.

AQUACULTURE

9. **U.S. aquiculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquiculture operation as a source for the virus?**

Data is inadequate to reach a conclusion

10. **It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquiculture and wild shrimp populations, with regard to shrimp viruses?**

I think that it is unsound to use such an analogy in regards to aquiculture. The experiences upon which' that conclusion is based comes from land based agriculture. Water, the universal solvent, provides a significantly enhanced transmittal medium and very different circumstances.

SHRIMP PROCESSING

11. **Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several" years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?**

Data is inadequate to reach a conclusion.

12. **Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?**

Yes

OTHER POTENTIAL SOURCES AND PATHWAYS

13. After considering the sources addressed in the shrimp virus report, what sources other than aquiculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

Bait shrimp and Non-Shrimp Translocated Animals (example: the growing culture of Australian red claw crayfish).

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

The testimony provided at the hearings appears conflicting on this issue. Until that can be resolved shrimp feed cannot be ruled out as a source.

STRESSOR EFFECTS

These next questions concern the possible consequences to wild shrimp populations and marine communities from exposure to pathogenic shrimp viruses.

15. **How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquiculture into the wild in South America?)**

There is no substantive evidence (which I have reviewed) that introduced viruses have had an effect on wild shrimp populations. Available information does provide evidence of transmittal of viral disease between wild populations and cultured shrimp. The evidence establishes a pathway, but does not contribute greatly to the assessment of risk.

16. **There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?**

As a significant data gap that must be addressed.

17. **How can changes in wild shrimp populations be used to interpret the effect**

(or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

Clearly, any population change (decline) outside norms would indicate an effect, although not necessarily from disease (hypoxia, el Niño effects, etc) would have to be accounted for and some empirical evidence would need exist for linking a decline to disease. Shrimp population models that adequately explain observed variability do not currently exist and until they do (even if possible) they will not be useful in this context.

18. **How important are potential viral effects on non-shrimp species?**

They can be very important, especially on susceptible species with low populations (ie listed endangered/threatened species) or with restricted distributions

COMPREHENSIVE RISK ASSESSMENT AND RESEARCH NEEDS

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to, the information presently available?

I am sorry, but "comprehensive" risk assessment is not defined in any of the supplied documents so I cannot ascertain what is contemplated. If you mean by comprehensive - taking a tiered approach and extending it beyond the qualitative levels into quantitative levels as new information is developed according to identified needs, then yes, that approach will make a positive contribution.

20. **What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp populations models), and what would be the likely time frame and cost?**

A quantitative assessment using shrimp population models would be useful if it were sensitive enough, but likely will not be timely or inexpensive. The taskforce report (page 53) estimates one year and $200-300K. That is optimistic at best and a case can be made that such a model would lack the sensitivity to meet the need. I lack the expertise to make such a judgement, but have some concern about it.

21. **Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?**

Yes, if 1 understand the question correctly this approach would likely give risk managers some better options to work with that they now have.

22. Summarize the critical research needs for completing such a risk assessment.

Three important research needs are: 1) *Assessing the presence and distribution of pathogenic viruses in wild stocks* - One "insufficiency in assessing the efficacy of disease management strategies is a lack of baseline information on the presence and distribution of pathogenic viruses in our native stocks. The recent occurrence of a "whitespot type virus in native species held in the Texas Agriculture Research Center in Corpus Christi illustrates that need; 2) *better information on infectivity, transmissibility and virulence of viruses* - one of the most immediate risk management needs is how can we minimize risk until some of the critical research needs are met. A more clear understanding of what is known about this topic and how that knowledge can be used to isolate cultured from wild shrimp is a critical management need; 3) *Assessing the relationship between stress and disease susceptibility in shrimp and evaluating the interaction among multiple stressors* - aquiculture conditions typically initiate stress sufficient to increase disease susceptibility and this is primarily due to over crowded conditions. If such conditions are not likely in wild populations can other stressors have a similar effect? 4) *Assessing the potential of shrimp processing activities in disease transmittal* - the risk we know the least about is that associated with the processing of imported shrimp. Based on sheer volume, it could overwhelms all others. Adequately assessing that risk will likely form the basis of future management strategies.

Wayne Munns

Shrimp Virus Workshop
Pre-Workshop Response to Questions
Wayne R Munns, Jr.

Management goals, assessment endpoints, and conceptual models

1. The draft management goal (p. 14 of the JSA Shrimp Virus Report) adequately captures two primary management concerns: 1) prevention of establishment of a potentially disruptive suite of viral agents in wild shrimp populations, and 2) minimization of the potential negative impacts on the sector of commerce involved with distribution of shrimp products to the North American market. A third management concern not addressed by the draft management goal might be stated as "minimization of potential negative impacts on resource populations and ecological systems other than wild shrimp". The focus of the draft management goal currently is limited to shrimp and the shrimp industry. Because the degree to which the viral agents can affect other species is not know with high certainty, some reflection of this concern maybe warranted.

2. The first assessment endpoint (p. 18) clearly reflects the first aspect of the draft management goal, and summarizes nicely the environmental value (and its attributes) of primary interest. A minor word smiting change maybe warranted, however. Strictly speaking, "populations" do not "survive, grow, and reproduce"; rather, these are attributes associated with individuals. Replacing the first occurrence of "of' with "in" would correct this.

   The second assessment endpoint, however, is less well crafted. It again focuses primarily upon shrimp populations, reflecting a focus on other ecology components only as support systems for the shrimp populations themselves. Effects on these support systems should be adequately reflected in the shrimp "survival, growth, and reproduction" attributes expressed

in the first assessment endpoint. As a corollary, it does not address potential effects on components of ecological systems which are more-or-less independent of shrimp populations, but which might represent high risk to these components. Inclusion of a third assessment endpoint addressing risks to non-shrimp components of ecological systems would be warranted given sufficient management concern (see Response 1 above).

3. My belief is that with the possible exception of the inclusion of a third assessment endpoint (see Response 2), the assessment should not be broadened to include stressors other than shrimp viruses, unless these other stressors interact with virus establishment, transport, and consequence pathways and processes. As communicated in the conceptual models described in the JSA report, pathways that to some degree reflect land use and production methods are considered, but only within the context of shrimp viruses. To broaden the scope to include other aspects of the shrimp industry would risk diffusion of the assessment effort.

Viral stressors and factors regulating shrimp populations

4. This question is difficult to answer. We know from other situations that predictions based upon exposure to stressors of naive laboratory test subjects often fail in validations against actual field situations. Pre-exposure to the stress can lead to compensatory responses (immunologic, homeostatic, and evolutionary responses) which reduce susceptibility to subsequent exposure. Recognition of this phenomenon (as well as the opposing situation of pre-exposure leading to enhanced susceptibility) will be important when identifying assessment uncertainties.

5. This is an area of obvious great uncertainty, and the answer to this question is critical to understanding the potential long-term consequences of virus establishment. That wild

shrimp populations occur in areas of the world in which shrimp viruses are indigenous suggests that some degree of immunity can be developed. The characteristics of these "compensated" populations, with respect to attributes such as productivity, stability, resilience, and susceptibility to other stressors, also is unknown. Also cogent is the time course of development of immunity. Although the potential development of immunity may minimize the long-term consequences of virus establishment in North America, the severity and extent of short-term ecological effects on shrimp populations maybe unacceptable from a risk management standpoint.

6. This will be difficult within the context of the risk assessment itself. As a data need, however, it is important to be able to separate the influences and risks associated with viral infection from other potential causes and stressors. Information regarding natural variability in the dynamics of wild shrimp populations, and the responses of those populations to anthropogenic stress, should be evaluated to provide expectations against which to overlay the effects predicted to result from viral infection. Further, the potential synergistic or antagonistic interactions between viral infections and other stressors represent a significant uncertainty for the assessment.

7. Statements to this effect are made in the JSA Report, but the data (as communicated) appear circumstantial at best, and precedents of "trans.-species jumping" by viral agents exist (ebola comes to mind). Although this likely is of minor management concern at the moment, further investigation of shrimp virus epidemiology as it affects humans may be warranted.

8. No.

Viral pathways and sources

9. As with potential risks to humans, little information exists regarding the epidemiology of shrimp virus transmission to wild shrimp populations. Although the lack of confirmed infection of wild U.S. populations would suggest a low probability of establishment from aquiculture operations, the data are too scant to evaluate aquaculture operations as a source of viral release. This represents a critical data gap in the aquiculture exposure pathway.

10. The potential transmission of viruses from domesticated animals to wild population likely is controlled in large part by three factors: 1) exposure of wild animals to domesticated animals and their by-products; 2) differences in the immunities of the two groups to pathogens; and 3) the frequency of infection in domesticated animals. The first factor is an explicit component of the conceptual model, and therefore will be evaluated as part of the risk assessment the second represents an important data gap; and we have data addressing the third. These factors will be explored as part of a risk assessment.

11. Little information exists regarding the epidemiology of shrimp virus transmission to wild shrimp populations. Although the lack of confirmed infection of wild U.S. populations would suggest a low probability of establishment from shrimp processing operations, the data are too scant to evaluate shrimp processing operations as a source of viral release. This represents a critical data gap in the shrimp processing exposure pathway.

12. The probability of release of viral agents as part of the distribution process likely is lower than that of the other pathways to be evaluated, but retail distribution as a potential source should be evaluated in the qualitative risk assessment.

13. An evaluation of existing data with respect to probabilities of transmission and establishment should be evaluated for all other sources (at least as identified in the JSA Report). Insufficient information is available to prioritize among these other sources.

14. Information provided in the JSA Report suggests that processing temperatures often are insufficient to kill viruses. Manufacture of shrimp feed should therefore be included along the pathways of shrimp processing and aquaculture.

Stressor effects

15. Such evidence provides direct information concerning the potential consequences of virus release and establishment in U.S. waters. Examination of shrimp populations in South America and Asia should provide useful data with which to bound the potential long-term consequences of viral infection. Cursory examination of that information suggests that because wild populations continue to exist, compensatory responses may occur that mitigate total devastation of those populations. Given the data at hand, however, it is impossible to determine the time course of such responses, and further to determine whether those populations are “impacted” relative to an uninfected condition.

16. This data gap is directly relevant to the issue of immunity and susceptibility of wild shrimp populations. As referenced in Responses 5 and 22, it is critical to understand whether immunity is a viable compensatory mechanism to mitigate the negative impacts of infection. As such, this will be an important source of uncertainty in the risk assessment.

17. Assuming that pathways can be established that link the release of viral agents with subsequent exposure to wild shrimp populations, and that infection of those shrimp can be documented, the responses of such populations can be used to predict (at least empirically) the responses of naive populations which might be exposed in the future. The time course of population change would provide information regarding the potential short-term consequences of infection, as well as provide indication of potential compensatory responses (e.g., development of immunity). Population modeling could assist in this evaluation in a number of ways, including: 1) supporting development of expectations of population dynamics (incorporating natural temporal and spatial variability) against which to evaluate short-term responses; and 2) providing predictive tools relating the biological effects of inflection to ultimate population response. The former application might require empirical evaluation of long-term data sets, whereas the latter would require mechanistic understanding of both direct viral influences on shrimp demographic characteristics (survival, growth, and reproduction) and potential compensatory mechanisms (e.g., immunity).

18. Unknown. This represents a critical data gap, particularly with respect to the third assessment endpoint suggested in Response 2.

Comprehensive risk assessment and research needs

19. The answer to this question will be determined in large part by the uncertainties recognized in the qualitative assessment we are about to conduct.

20. The answer to this question will be determined in large part by the uncertainties recognized in the qualitative assessment we are about to conduct. A more comprehensive risk assessment could incorporate quantitative estimates of the probability of virus transmission, as well as quantitative models of both viral and shrimp population dynamics.

21. Should the initial qualitative, or subsequent more quantitative assessments suggest that the risks of establishment and the consequences of establishment be unacceptably high, then an assessment comparing various mitigation options (including treatment options) may be warranted.

22. Assuming the question to refer to a comprehensive risk assessment, the critical research needs from my perspective include concrete information concerning:

    1. potential compensatory responses (e.g., development of immunity) of wild shrimp populations exposed to the viral agents, including insight into the time course(s) of such responses

    2. susceptibility of non-shrimp native species to viral infection and the consequences of such infection

    3. the basic epidemiology of shrimp virus disease transmission, including identification of potential intermediate vectors, natural attenuation rates, etc.

    Additionally, diagnostic methods for surveillance of shrimp viruses in wild populations are needed to establish current and future levels of infection. Such data would help to address the three research needs identified above.

Gary Pruder

Dr. Gary D. Pruder, VP
The Oceanic Institute
U.S. Shrimp Farming Program

Premeeting Comments
Shrimp Virus Peer Review

1. Management Goal: *Prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing and aquaculture operations.*

The introduction of disease causing viruses to shrimp farming operations has been shown to have immediate and drastic impact. Suggest that the management goal be expanded to exclude the introduction of desease causing viruses to shrimp farms.

2a. Assessment Endpoint (1): *Survival, growth and reproduction of wild shrimp populations in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters.*

In keeping with #1 above, suggest that survival and growth of shrimp in farms be added as assessment endpoints.

2b. Assessment Endpoint (2): *Ecological structure and function of near shore marine communities as they effect wild shrimp populations.*

Perhaps such an effort is out of reach.

3. *It has been suggested that the scope of the proposed assessment be broadened to consider the impacts of alternative land use and seafood production methods in coastal areas.*

Seafood production methods will likely be included in preventing the introduction of viruses. Recommended against expanding the scope to include other environmental impacts at this time.

4. *How relevant to virus effects on wild populations is information on infectivity and effects that are derived from laboratory or intensive aquaculture operations?*

Likely that information from laboratory and shrimp farming operations will represent worst case scenarios in individual mortality and survival percentages.

Dr. Gary D. Pruder

5. How likely is it that exposure of wild shrimp to viral diseases could lead to development of immunity and reduced effects on population survival over time?

It appears to be a reasonable course of events. It would be valuable to know what genetic changes if any, accompany increased resistance, if any, to the disease agents.

6. How can strong influence on both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

Sometimes but not often. The systems and the interactions are complex and do not lend themselves to controlled experiments..

7. Can human health effects from shrimp viruses be ruled out as a concern?

Not sure

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

Probably yes for some viruses and unsure for others.

9. U.S. aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support of refute the importance of aquaculture operations as a source for the virus?

Aquaculture operations do not create viruses. However, if a farm becomes infected it is likely that the virus will be multiplied and subsequently be transferred with shrimp product, shrimp waste and/or discharge waters. Presently, high health shrimp farms are subject to infection transfer from wild animals. It is critical that steps are taken to exclude viral diseases from shrimp farms.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animals; usually it is the other way around. How well does this observation apply to the relationship betwecn shrimp in in aquaculture and wild shrimp populations, with regard to shrimp viruses?

Perhaps not too well. The differences between domesticated shrimp and wild shrimp and not yet substational. Our experience to date in breeding shrimp, has indicated that wild shrimp are more resistant to many stresses including disease.

Dr. Gary D. Pruder

11. *Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign countries for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?*

It is only recently that virus related problems were recognized as serious problems by foreign shrimp producers. It is unlikely that shrimp processed over the past twenty years carried significant viral infections. However, those processed over the last three or four years are known to carry high levels of virus.

12. *Should retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?*

The practice of selling older shrimp products as bait should be discouraged.

13. *After considering the sources addressed in the shrimp report, what sources other than aquaculture and shrimp processing are most critical for evaluation in risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?*

Live shrimp and bait shrimp are likely carries of shrimp viruses and potential transfer products.

14. *Is manufactured feed a potential virus source, or is the processing temperature sufficient to rule this source out?*

It is unlikely that feeds are involved in the current problem. I do not know about temperature.

15. *How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted.*

No comment.

16. *There is presently a lack of basic data on background levels of pathogenic viruses on wild shrimp populations in U.S. waters. How should this data gap be evaluated in risk assessment?*

Recent findings confirm the presence of exotic viruses in wild populations. The real issue goes back to #15.

Dr. Gary D. Pruder

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp models be used in the future?

No coment.

18. *How important are potential viral effects on non-shrimp species?*

Direct economic impact would be much less. Do not know about how long term indirect impacts.

19. *How will a comprehensive risk assessment contribute to management of shrimp virus problems?*

The assessment will organize existing information. Hopefully it will also support the need for research to fill information gaps.

20. What type of assessment should be conducted next and what would be the likely time frame and cost?

Suggest a combined modeling and multiple case study be undertaken to set some reference points. I suggest $15 MILLION over the next three years. In the meantime, aquaculture and processing operations should be assisted in developing economic methods to disinfect both incoming and effluents.

21. *Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?*

Development of treatment options should be undertaken immediately

22. Summarize the critical research needs for completing such a risk assessment?

Suggest we follow the data gap and research need recommendations page 49-51 of the Evaluation Report by the JSA shrimp Virus Work Group

Paul Sandifer

Responses from Paul A. Sandifer, SC Department of Natural Resources

Management goals, assessment endpoints. and the conceptual model

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

The goal is very clear and does a good job of incorporating most of the elements of the problem. However, I recommend the following minor modification suggested changes noted in bold):

> "Prevent the establishment of new disease-causing viruses in wild populations of **penaeid** shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing possible impacts on shrimp importation, processing, aquiculture operations **and the ecosystems upon which wild penaeid shrimp stocks depend."**

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger ecological system or, alternatively, to the aquiculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

I think that the emphasis of the risk assessment should remain on penaeid shrimp, but other information should be included where it is available and pertinent. However, the available information on the occurrence and impacts of various viruses in penaeid shrimp populations is very sketchy at best, and that for other organisms appears to be extremely limited. Nevertheless, a minor modification of the second assessment endpoint as noted below (suggested change in bold) might be helpful, since it would not limit the assessment of ecological effects to just those dealing with marine shrimp populations:

> "Ecological structure and function of coastal and near-shore marine communities, **especially** as they affect wild penaeid shrimp populations."

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

I am adamantly opposed to much broadening of the risk assessment, because I believe such would result in the EPA's inability to draw any useful conclusions within a reasonable time frame. Broadening the scope of the assessment to include other areas with very limited data pertinent to the occurrence and impacts of shrimp viruses would needlessly complicate the process and, in my view, likely ensure its failure.

Paul A. Sandifer

Viral stressors and factors regulating shrimp populations

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived form laboratory or intensive aquiculture operations?

Very relevant, since in most cases this is the primary information we have about potential pathological effects. However, this question could probably be better addressed by epidemiologists with experience with viral diseases of arthropods (e.g., insects). Information from other better known situations, such as some virus diseases of insects or domesticated animals or plants might prove very enlightening.

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduces effects on population survival over time?

It is quite possible that effects in wild populations (and probably cultured populations as well) might diminish over time with repeated exposures. Whether or not such diminution would be the result of an acquired "immunity" or some sort of accommodation (see Flegel and Pasharawipas, viracom 23 June 97) is unknown. Also unknown is how long it might take for wild populations to develop such protection, if at all, and the possible effects on survival of the wild stocks until such accommodation occurred.

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

One would have to look very carefully at long-term data series on shrimp populations and then attempt to correlate population level effects (if any) that were greater than those associated with "normal" environmental variation and persistent.

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

I would leave this to those with expertise in human health in relation to virus diseases.

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

NO.

Viral pathways and sources

9. U.S. aquiculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquiculture operations as a source for the virus?

In most situations it does neither, since there are few if any baseline (before aquiculture) data on the incidence (if any) of viral infections in wild shrimp populations for comparison, and little if any work has been done to determine if archived samples such as in museum collections could be analyzed in any way to provide such “before” data.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquaculture and wild shrimp populations, with regard to shrimp viruses?

I am not sure. It is clear that aquaculture operations have spread viral diseases from one facility to another, and they may well have spread viruses to wild shrimp populations, but documentation of this latter appears to be lacking. Again, the lack of baseline data on the occurrence of viruses in wild shrimp populations, and indeed the distribution of viruses in wild crustaceans worldwide, makes it difficult to draw many conclusion. Further, at least in the US, it is my impression that relatively little sampling has been done of wild shrimp populations, even around aquiculture operations, for viral analysis, and what analyses have been done have generally followed disease outbreaks in the aquaculture operations. Thus, it is difficult to determine in many situations whether the disease came to the aquaculture operation from the wild or whether the aquiculture operation introduced the disease to the wild.

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

I have not seen enough data from analyses of virus incidence in local wild shrimp populations to draw any conclusions in this matter.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

Yes. It is my understanding that some shrimp are harvested from apparently diseased ponds in South America at very small size and then packaged whole in bags for direct sale in the US for fish bait. The only processing these shrimp undergo is external washing, packaging in small plastic bags, and freezing. Many other shrimp products come into the US with the potential to be carrying viral diseases and go directly into wholesale and retail distribution networks, with little or no additional processing and certainly none that would affect the viability of any viruses they may carry.

13. After considering the sources addressed in the shrimp virus report, what sources other than aquiculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

The most important other potential source of some virus infections that deserves considerable discussion at the workshop, in addition to aquaculture, shrimp processing, shrimp importation and retail sales, is the local wild stocks themselves. Evidence is mounting that there is widespread occurrence of a “white spot complex virus" in crustaceans, including penaeid shrimp, in US South Atlantic waters, and that this virus has moved from the wild into culture facilities. Whether the virus has been in the wild for a long period of time or was introduced only relatively recently needs much further study. It may well be that there are a number of viruses naturally occurring in native wild shrimp populations, and that these could affect aquiculture operations and/or wild populations.

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this out?

I believe that feed should be considered a potential virus source until ruled out by testing for viable virus particles. Not all feeds provided to aquiculture operations in this hemisphere are likely to be processed at high temperatures, and it is quite possible that some lots fail to get cooked as much as they should. Experimentation should be undertaken to resolve this question. For example, one might incorporate some shrimp tissue known to be infected with virus into the shrimp feed preparation and then process it as normal. The final product tested would then be tested for the presence of viable virions.

Stressor effects

15. How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquiculture into the wild in South America?)

I have seen no evidence that conclusively links an outbreak of virus disease in aquiculture operations with failures of a local wild stock, although the potential for such effects certainly appears to be present. The problem with the correlation of IHHNV with the decline of the *Penaeus stylirostris* fishery in the upper Gulf of California is that it was a single factor correlation, and other potential contributing factors apparently were not taken into consideration. At this time, it seems impossible to determine just how much, if any, of the problem in that fishery was the result of IHHNV. The situation with regard to TSV in wild stocks in South America is even more confusing. It appears likely that the virus was spread by shrimp farms, but it originated from the wild somewhere, perhaps in South America, perhaps elsewhere. Clearly the virus is widespread now in wild stocks in much of the region, but I do not know if there is

sufficient evidence to determine whether it existed in these same stocks prior to being observed on shrimp farms or not. Also, I am not aware of whether there are data on the wild stocks, either from the fisheries themselves or from fishery-independent surveys, that suggest any collapses of local populations in association with observations of the virus in the wild.

16. There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?

I believe that an immediate effort must be made to at least partially fill this data gap before any realistic assessment of risk can be completed. This is probably the most pressing need.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

This will be very difficult over the short term. Wild shrimp populations are notoriously variable, primarily in response to environmental factors. Unless one sees something like a catastrophic decline in population abundance at the same time that environmental factors are considered "good" for shrimp — and one has reliable data on incidence of one or more viruses in the wild population, with associated and evident pathology — it will be very difficult to draw firm cause-and-effect conclusions. It may be possible to use one or more of the existing empirical shrimp population models to estimate an effect of a virus outbreak in a wild population, if the model has a good track record of predicting effects of environmental factors and then something occurs in the population that makes the predicted value considerably different from the observed. At best, however, this would be an indicator, not a clear signal of cause.

18. How important are potential viral effects on non-shrimp species?

Very, but they may be difficult to evaluate in the short term.

Comprehensive risk assessment and research needs

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

I do not know if it will add to the information available, but it will certainly result in a synthesis and assessment of the currently available information that will be of great use to many involved with the shrimp virus probIem. Agencies such as the one I work for (the SC Department of Natural Resources) will undoubtedly use the risk assessment in formulating regulatory policy and setting priorities for research, development and management activities.

20. What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp population models), and what would be the likely time frame and cost?

Quantitative risk assessment is clearly needed, but much more data than is currently available will be needed before beginning such. A badly flawed quantitative assessment based on poor data would likely do more harm than good. I have little experience in this area, but would guess that a minimum time frame would be 5 years, with a cost on the order of $5-10 million over that period.

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

Yes. As much as possible, note of such treatment options should be included in the present qualitative risk assessment.

22. Summarize the critical research needs for completing such a risk assessment?
A comprehensive evaluation would take much more time than I have at present, but the following are some of the most pressing needs.

a) Further refinement, testing and validation of diagnostic techniques for the viruses in question, coupled with development of more user-friendly techniques that could be used on a broad range of kinds and numbers of samples.

b) Development of a reliable and detailed data base on the incidence and effects of viruses in wild shrimp populations and populations of other near-shore and coastal crustaceans. This should include identification and examination of archived samples from as many years ago as practical.

c) Development, testing and demonstration of reliable and cost-effective methods for treating infected aquiculture facilities, including large outdoor ponds, to eradicate shrimp viruses and prevent escape to the environment:

d) Based on studies from other fields (e.g., insect population studies), as well as direct observation and carefully crafted experiments, determine the likely effects of shrimp viruses in wild populations.

e) Experimentally evaluate the potential for acquired “immunity” or accommodation to the viruses in question by captive shrimp.

f) While not a research issue per se, one of the most pressing needs is for a standardized process and bureaucratic mechanism for inspection and certification of brood and seed stock shrimp for distribution around the country to aquiculture facilities.

Max Summers

Premeeting comments are not available at this time.

Suzanne Thiem

Comments on the Shrimp Virus Report

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Management goals, assessment endpoints, and the conceptual model

1. The stated management goal is “prevent the establishment of new disease-causing viruses in wild populations of shrimp in the Gulf of Mexico and southeastern U.S. Atlantic coastal waters, while minimizing the possible impacts on shrimp importation, processing and aquiculture operations.” From the material presented in the report this goal seems to be too narrow. It appears that the presence of diseased shrimp in aquiculture ponds and importation and processing of diseased shrimp, in particular, could negatively impact native shrimp populations in many ways. A broader statement, such as “Maintain the health and ecology of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. coastal waters, . ..” would encompass non-viral shrimp diseases as well as other stressors.

2. The assessment endpoints established for this report are:
Primary: “Survival growth and reproduction of wild penaeid shrimp populations in the Gulf of Mexico and southeastern U.S. coastal waters.”
Secondary: Maintain? Preserve?.’’Ecological structure and function of coastal and near-shore marine communities as they affect wild penaeid shrimp populations.” (this is not a sentence)
The primary assessment endpoints seems appropriate at this time since it should be a reasonable indicator of the impact of viruses as well as other stressors on natural shrimp populations and provide at least minimal feedback on the health of the ecosystem. However if shrimp population declines are observed, this endpoint can not distinguish if virus infection is the reason for the decline. As for the second endpoint, I’m not sure how it could be measured.

3. I would agree that the scope is probably too narrow, even if the primary concern is the health of the native shrimp populations and/or other fauna. In addition to the issues of other shrimp diseases and exotic shrimp species- other factors impacting coastal waters such as development and seafood production certainly should be considered since they can effect nutrient and oxygen levels in the water, temperature, etc. If the shrimp or other organisms

are stressed they also may become more susceptible to diseases including introduced or naturally occurring pathogens.

Viral stressors and factors regulating shrimp populations

4. Studies were cited about the transmission of several of these viruses to different shrimp species and to other crustaceans as well as other arthropods, yet without further knowledge of how these transmissions were evaluated, it is impossible to judge the value of these results for risk assessment Specifically, it is often, possible to transmit a disease in laboratory situations but not in a natural situation. Thus, in the natural environment it is not clear how susceptible native shrimp species are to the viruses infecting non-native shrimp species. Also with a few exceptions, viruses tend to be specialized, generally having relatively narrow host ranges. However, since these shrimp species are related they may well be susceptible and possibly even more sensitive to viruses from other locales. Without evaluating the methods used to obtain the data, in particularly how the virus input and virus from the resulting infections were validated, I am suspect of reports of transmission to other organisms such as crabs. Laboratory results can certainly give baseline data and in particular demonstrate if transmission is possible- but can not accurately predict outcomes in natural situations. Likewise an intensive aquiculture operation is quite different from a natural situation. For example, to become infected a shrimp would have to encounter the virus, yet we don't know the distribution of viruses in the natural habitat or how likely it would be for the host to come in contact. In an intensive aquiculture system, the spread of viral disease is greatly enhanced.

5. I don't know if shrimp can or will develop "immunity" to virus diseases- little is known about immune responses of invertebrates to viruses and they lack the immunological memory of vertebrates. However it is possible that resistant populations will develop.

6. Other stressors surely have an impact on shrimp populations and I believe it will be difficult to separate the impact of these factors from the risks of viral stressors.

7. It is highly unlikely that these viruses can effect human health. Viruses co-evolve with their hosts and become highly adapted to particular hosts. Given the tremendous evolutionary distance between vertebrates and invertebrates (approx. 540 million years) it is improbable that these viruses could infect humans or other vertebrates even by mutating.

8. I am not familiar enough with the identification techniques used for identifying these viruses to make a judgment on their reliability.

Additional comments on viral stressors: Are these viruses really new? These viruses are described as new or exotic throughout the report. However, from the material presented I'm not convinced that similar viruses are not already present in native shrimp populations, but data to support or refute this idea are lacking. If some of these viral diseases are detected in native populations how will we know if we are detected a domestic cousin-or an exotic variety? Viral disease outbreaks can be expected to occur when populations are crowded since virus levels can be amplified and spread, as they have in aquiculture operations in Asia and South America. If native species were grown in high density aquaculture, disease outbreaks from native pathogens would be expected, particularly if appropriate sanitary/hygienic procedures were not routinely applied.

Viral pathways and sources
Aquiculture

9. There is not sufficient information on virus infections in wild shrimp populations to support or refute the importance of the aquiculture operations as source of virus infection in wild populations. However, aquaculture is one of the most likely potential source for virus inoculum because large amounts of virus can be produced during disease outbreaks. In addition, other diseases such as bacterial, fungal, or rickettsial diseases, have the potential for adversely affecting native shrimp populations as much as viral diseases. Again high density aquiculture could provide a means of amplifying these diseases as well and increasing the risk of their spread to native populations.

10. There is not enough information to determine if shrimp in aquiculture can infect wild populations. The two most important factors for the infection of wild animals by diseases of domestic animals am the probability of exposure and susceptibility to the disease agent. In the case of shrimp neither of these parameters are well characterized.

Shrimp processing

11. There is insufficient information to support or refute the claim that processing virus-infected shrimp is a source for viruses infecting native populations. However the practice

of some shrimp producers to harvest and ship diseased shrimp makes this one of the more Likely sources for virus contamination of native shrimp populations.

12. It seems less likely that shrimp in the retail distribution system would be a substantial source for virus exposure of native species than aquaculture or processing since it would be less likely that viruses from this source would enter the coastal waters.

Other potential sources and pathways

13. Of the other sources mentioned in the report, bait shrimp and ballast water are the most likely virus sources that could impact native populations. However unlike aquiculture and shrimp processing operations that process imported shrimp that may be diseased, virus levels from these sources are unlikely to be as high.

14. Not enough information on the manufacturing of shrimp feed was given to evaluate its potential as a virus source. The report stated that shrimp meal was not heated enough to kill viruses and it was added to feed. But it is not clear how extensive the use of this shrimp meal is for feed stock for shrimp aquaculture. In any case, it would impact aquaculture primarily. Thus, its impact would be secondary- increasing infection rates in aquiculture leading to greater risk of exposure of native species from this source (see #9).

Stressor effects

15. What is the impact of shrimp viruses arnpli.tied in aquiculture on natural populations in Asia and South America? Since these viruses are pathogens of the native species, I would expect that if there was significant transmission of disease from aquaculture (or other sources) to native populations it would be observed in these situations resulting in greater mortality from virus than would normally be observed in the absence of aquiculture operations. The one cited example of shrimp decline from IHHNV in the Gulf of California was disputed by Dr. Alvarez, Instituto National de la Pesca, Mexico, who suggested other causes for the decline. Another report by C. R. Laramore on viruses in native shrimp populations in Honduras following TSV outbreaks in aquiculture showed no noticeable effects on the native populations. These data are not sufficient to make any conclusions on the effects of introduced viruses on native populations. Both are correlative but not conclusive.

16. Due to a lack of knowledge about native pathogenic viruses, I would approach the risk assessment conservatively by assuming a minimal impact of native viruses until more data is available. Thus until it can be shown otherwise assume virus infection observed is from introduced viruses. That way risks from introduced viruses would be less likely to be underestimated.

17. Shrimp population data could be used in monitoring the overall health of the shrimp populations, but additional data on virus loads within the population is needed to make any congelations with virus impact To get a good handle on the effects of virus vs. a multitude of other stressors a database should be developed over time that includes populations, pathogen loads from sampled specimens, and physical data such as temperatures, dissolved gases, etc. This type of data maybe currently available saris the virus loads. This would help determine the impact of various factors on shrimp populations and make it possible to develop shrimp population models that could be used to more accurately evaluate the effects of different stressors including viruses.

18. Shrimp viruses could impact non-shrimp species in two major ways. First, shrimp are an important link in the food web, severe losses of shrimp from virus infection (unless other species fill their niche) would impact shrimp predators. Secondly, if these viruses do indeed infect other species they could have a direct impact on these species. It is difficult to judge how big or important these impact would be since it would depend on the extent of the viral disease and the magnitude of the loss. Again there is a major data gap on how these viruses are transmitted in natural conditions as well as their persistence in the environment..

Comprehensive risk assessment and research needs

19. A comprehensive risk assessment is a good idea. Clearly shrimp viruses, previously identified in foreign aquiculture operations, are present in both domestic aquiculture operations and in imported shrimp indicating that they area potential risk to native shrimp in our coastal waters. A significant problem in assessing the magnitude of the risk is the lack of good data on a number key issues. A comprehensive risk assessment will serve to identify and prioritize these gaps. As I see it the greatest uncertainties are biological, particularly as it relates to exposure of native populations and possible establishment of these viruses in the wild.

20. I'm not sure what retrospective data is available on native shrimp populations, but it would appear to be necessary to draw any conclusions about the impact of viruses vs. other stressors on shrimp populations. I'm also not sure what type of model couId be developed with so little knowledge of the virus distribution and life cycle. Cost? I don't have the experience to begin to estimate the cost of such an assessment A tiered assessment might be a more reasonable approach. That way qualitative estimates of risk could be used until sufficient data were available to get a better quantitative risk assessment.

21. It would be prudent to consider treatment options to reduce the risk of exposure.

22. In my estimation the, most critical research needs for a risk assessment for exposure to shrimp viruses are their likely impact on shrimp populations: 1) Determiningg the likely chance for exposure in the wild from any exogenous virus source. This would include the fates of viruses that are released into environments, such as likely location in the water column with relationship to locations of susceptible shrimp populations, the length of virus viability in natural habitats, and the viruses mode of entry into hosts. 2) Determing the susceptibility of native shrimp species under natural conditions, including which developmental stages are most susceptible. 3) The nature and extent of viruses in wild shrimp populations in coastal waters?, including "native" and putative introduced viruses needs to be assessed. This may require development of new diagnostic and survey techniques.

Other comments : If viral pathogens in insects are used as a model for shrimp viruses, disease outbreaks (epizootics) are generally cyclic are correlated with high insect population densities. Because viruses are obligate parasites their levels can only increase when they infect a susceptible host. Viruses in the environment are gradually inactivated so that only low levels remain. Therefore the probability of an insect encountering an infectious virus is low and if an insect does become sick and die, the probability of another susceptible insect encountering the diseased insect or amplified virus is also low. However, when host densities are high the insect that is infected by chance encounter and becomes sick will be in close contact with additional susceptible insects which allows the virus to be amplified and spread extensively within that population leading to a population crash and the deposition of large quantities of virus in the environment. Release of high amounts of virus (naturaIly or artificially) into the environment increases the chance that a susceptible host will come in contact with the virus and become infected even at low host densities.

Gerardo Vasta

SHRIMP VIRUS REVIEW WORKSHOP

(A) MANAGEMENT GOALS, ASSESSMENT ENDPOINTS AND THE CONCEPTUAL MODEL

(1) **How does the management goal reflects the dimensions of the shrimp problem?** Overall, the management goal reflects quite adequately the dimensions of the shrimp problem to be addressed in the short term. The proposed ecological risk assessment conceming shrimp viruses is appropriate because the potential threats to the natural ecosystems and the shrimp industry are both serious and urgent. These potential threats to US native wild shrimp populations from nonindigenous shrimp viruses arises from Possible escapes of imported shrimp and insufficiently treated effluent from aquaculture facilities, shrimp processing solid waste and effluents, scavenger sea birds, bait for recreational fishing, human waste/sewage, ballast water and others. It is also well documented that under both experimental and natural conditions, shrimp viruses can infect various shrimp species and other crustaceans. Therefore, the potential for transmission of these viruses, principally from aquaculture and processing operations to native wild crustacean populations, although not yet well documented, should be a serious concern.

(2) Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system, or, alternatively, the aquaculture industry. Please, comment on the assessment endpoints as the focal point for the ecological risk assessment The potential negative effects of viruses on shrimp wild populations, organisms other than shrimp, and the ecosystem as a whole, that may result from the aquiculture and processing industries and other factors, are relevant and worth addressing with urgency, In fact the consensus among the environmentalists seems to be that protection of wild shrimp must take precedence over shrimp aquaculture, and clearly, a substantial industry in the Gulf coast is based on domestic shrimp fisheries, However, the success of imported shrimp processing and mariculture operations in satisfying the consumer demand for shrimp (70-80% of the shrimp market), may alleviate the pressure on wild shrimp populations, food webs and the ecosystem as a whole. Furthermore, it should be considered that many marine ecosystems have been transiently or permanently damaged by commercial fishing practices, and current shrimp fishing methods may have similar environmental effects. Because of greater efficiency and potential to control its environmental effects, food farming is now preferred to food capture. Thus, the

risks associated with shrimp viruses on wild shrimp populations, shrimp mariculture and the ecosystem as a whole should eventually be assessed as an integrated initiative. What is badly needed are (a) the resources to conduct monitoring at the three assesment points (b) the tools to carry out the monitoring (C) to interpret the data as a coordinated effort in order to truly uinderstand the sources and pathways of the disease agents. In the long term, US native species may be selectively bred and genetically improved to become useful mariculture species, avoiding the need of farming nonindigenous species. In fact there is a precedent of this possibility in the attempts to farm *P. setiferus* in Texas.

(3) **It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.** If the risk assessment does not address the need to preserve and improve coastal current mariculture operations, we should be prepared to accept the risk of increasng alternative food production methods,, such as shrimp trawling with the associated fish and turtle kills and high pressure on the wild shrimp populations and, ultimately, on the food webs. If coastal shrimp farming is to be stopped alternative agricultural land uses that would produce runoffs with fertilizers or chicken/pig feces could have serious environmental impacts such as the algal blooms, including *Pfiesteria piscicida* outbreaks observed on the Atlantic coast Any use of coastal land will have an impact on the coastal marine ecosystem and appropriate land use polices, such as the establishment of buffer zones, and rational management practices should be developed m order to minimize the impact.

(B) VIRAL STRESSORS AND FACTORS REGULATING SHRIMP POPULATIONS

(4) How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquaculture operations? The contributions of scientific research to several of the issues under consideration, represent the only body of evidence on which a solid base for a risk assessment initiative, and clearly indicate that this information is not only is very relevant but much more of it is needed to elaborate a useful risk assessment. Needless to say that like in both the laboratory or pond setting, experiments have to be correctly designed, adequately controlled, and the data interpreted with caution. In the absence of reliable field

data on wild shrimp populations, the aforementioned experimental approach sometimes constitutes the only source of knowledge we can rely upon.

Although the laboratory conditions may not exactly replicate the changing environmental conditions, most variables can be manipulated and controlled in a way that even those environmental conditions that are not very frequently observed can be simulated. The resulting data can then be used to gain insight in problems of infectivity of nonindigenous viruses for native shrimp species in the environment Native species such as *P. setiferus, P. aztecus* and *P. duorarum* can be infected experimentally with IHHNV under laboratory oonditions, by injection or by offering virus-infected tissues as sole food source. Experimental studies demonstrated that *P. setiferus,* but not *P. aztecus or P. duorarum,* could be killed by TSV. Furthermore it was concluded that the three US. native species can serve as carriers or reservoir hosts of TSV without necessarily exhibiting disease (Oversheet et al, 1997). Although disease or mortalities did not necessarily occur in all the experimental animals all, therefore, it cannot be concluded that infection, disease or mortalities will happen in open waters, the potential risk of this event taking place cannot be ignored. Infection or a carrier status, should be considered a determinant factor that underscores the possibility that these viruses may have detrimental effects in native shrimp species and the environment overall. Stressful environmental conditions affecting infected, although not diseased, shrimp may determine different outcomes. Additionally, mutation of the established virus may lead to more virulent strains in an unpredictable manner. The genetic susceptibility of cultured *P. vannamei* to infectious HIINV and *Baculovirus penuci* has been recently examined arid the possible relationship with growth status and metabolic gene expression characterized (Alcivar-Warren et al, 1997). The transmission of viruses in the wild shrimp populations is a documented faet and experiments an be designed to determine the viral doses that may lead to infection in open waters. Therefore, the laboratory experimentation has revealed the potential threat of exposure of native species to nonindigenous viruses, and it should be considered as the first step of a process that generates the scientificc knowledge necessary to develop risk assessment and management Strategies.

Results obtained from intensive aquaculture operations are very rclevant, particularly in the absence of detailed field information on the wild populations. Although the aquaculture setting, particularly under high density rearing, is stressful in nature, it is important to understand the potential risks for the native species under those stressful conditions. For example, pond trials have yielded controversial results concerning the risk

of TSV infectivity for native species, such as *P. setiferus,* as compared to *P. vannamei..* In some studies, *P. setiferus* was not affected by the presence of TSV-surviving *P. vannamei* or by the presenee of TSV-infected P. vnnamei in adjacent ponds. Studies on the influence of salinity on the susceptibility of fanned P. *vannamei* to TSV, and the impact of aquaculture on wild shrimp populations in Honduras, illustrate how intensive aquaculture operations maybe used to gain insight in viral infection and disease. However, additional experimentation under controlled conditions in the laboratory end intensive aquaculture operations will be necessary to establish the risk involved in cross-species infectivity of nonindigenous viruses and disease.

(5) How likely is that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival overtime? It has been shown that some short term immunity in arthropod species ccn be induced by challenge of with non-self materials, but overall invertebrates are not endowed with immune memory and neither permanent nor long term immunity has been demonstrated so far. Invertebrates lack a B cell/T cell/immunoglobulin-mediated adaptive immune system, but are able to recognize and respond to non-self substances at least as efficiently as vertebrates do. Invertebrates rely on non-specific innate mechanisms that although may be inducible, only result in short-lived responses that in most cases do not discriminate between individual pathogens. Therefore, responses mounted by invertebrates to potentially infectious agents are mediated by immune systems only m the sense that they resemble qualitatively the "innate" or, "natural" immune responscs of vertebrate myeloid ccllsand non-imrnunoglobulin, humoral components. Passive immunization with rabbit antibodies against a luminescent *Vibrio harveyi* strain 820514 originally isolated from diseased *P. monodon,* has been recently studied and results suggest and enhanced disease resistance in the treated animals for the first two weeks (Lee et al 1997).

Invertebrate defense responses exhibit common themes such es phagocytosis and encasulation, but the underlying molecular recognition and effector mechanisms can be considerably diverse. The best characterized components of immunity in the crustacea are the glucan-binding proteins and lectins as recognition molecules, and the prophcnoloxidase system and antibacterial peptides as effecter factors. However, is not yet clear how the various components interact in the internal defense system against viruses. Some of the factors involved, such as a-2-macroglobulins, C-reactive proteins, antibacterial peptides, serine proteinases and proteinase inhibitors have been substantially conserved through the

evolutionary lineages leading to the chordates, whereas others, such as C-type lectins and complement-related factors, only retained those regions of the molecule or single amino acid residues that are relevant to recognition/effector functions. Finally, for other factors such as glucan-binding proteins and some antibacterial peptides from crustacea, no homologues have been identified in vertebrates so far, and appear to be exclusive of invertebrate species. Penaeidins, a new family of antimicrobbial peptides isolated from the hernolymph of *P. vannamei,* ***has*** been recently described (Destoumieux et al, 1997)

In addition to phagocytosis, encapulation and nodule formation can be observed in the crustacea Pathogens often elicit encapsulation with consequent inactivation or death of the invader through toxic intermediates from an enzymatic cascade pathway that results in melanization. The recognition/effector rnechanism responsible is the prophenoloxidase activating system, that is present in most invertebrates and contains factors that are directly involved in communication between invertebrate hemocytes. A plasma recognition protein binds the polysaccharides or glycoproteins on the pathogen surface and induces activation of a prophenoloxidase-activating enzyme that will cleave the proenzyme prophenoloxidase to yield phenoloxidasc. This active enzyme will catalyze the oxidation of phenols to quinones that will polymerize and form melanin, all exhibiting anti-rnicrobial properties. In the shrimp *P. paulensis,* the great majority of the prophenoloxidase activity is found in shrimp bemocytes, is cation (Ca, Mg)-dependent and is enhanced by microbial cell wall components such as LPS and ß1-3 glucans suggesting a role in non-self recognition. Associated factors involved m cell adhesion and degradation are also present (Perazzolo and Barraco, 1997). The interaction of hemocytes with foreign materials can further trigger clotting of body fluid (i.e. plasrna) that would aid in internal defense by blocking or slowing the spread of microbes in the body cavity. Among the non-self recognition molecules, members of the immunoglobdin superfamily have been clearly identified in arthropods. However, only hemolin, a protein isolated from insects, can be induced upon pathogen challenge. Lectins (carbohydrate-binding proteins) are widespread, usually constitutive or inducible, components of invertebrate body fluids and tissues. CommonIy multivalent, these molecules can aggregate microbes with the appropriate saccharide moieties on their surfaces. Simple aggregation of microbes can aid internal defense by restricting the distribution of potentially pathogenic agents and promote their phagocytosis. Such opsonization maybe the result of conformational changes on the lectin upon binding to ligand that arc recognized by the phagocytes.

Because it is unlikely that true immunity will be induced by exposure to the viral pathogen, reduced effects on population survival cannot be expected. At best the continued impact of a viral pathogen on the shrimp populations could lead to selective survival of disease-resistant individual, strains or races. In the Gulf of California the wild *P. stylirostris* shrimp populations rebounded from the presumed IHHNV-caused mass mortalities, to harvestable levels after six years. It would be very interesting to examine if the current shrimp populations in the Gulf of California are equally or less susceptible to IHHNV than other populations from locations that have not been exposed to the disease.

(6) How can the strong influence of both natural and non-viral anthropogenic factors on shrimp popnlations be separated from risks associated with viral stressors?: It is possible that changes in salinity, and temperature, heavy metals or other pollutants, such as fertilizers in run offs that cause eutrophication of the environment could stress coastal or estuarine shrimp populations and increase their susceptibility to viral disease. In the case of the Mexico's Gulf of California some evidence points to an association between detection of IHHNV in wild *P. stylirostris* shrimp and a decline in those populations, but other environmental factors may have compounded the problem Further, it has been proposed that overfishing may have significantly contributed to the decline. Basic laboratory studies on effects of environmental factors such as temperature, salinity, heavy metals on the immune capabilities of shrimp are urgently needed in order to gain insight in the risks of climatic changes, such as El Niño, or anthropogenic factors on shrimp viral disease. Similarly, the recovery of the populations may have been due to either the return to "normal" environmental conditions, or the selection of shrimp races or strains with enhanced disease resistance. Therefore, although experimental research can provide valuable information on the effect of each environmental variable on shrimp susceptibility to disease, it may be difficult to separate these factors from the risks associated with viral stressors, without oversimplifying the problem.

(7) Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?: In general virus that infect invertebrates do not infect mammals and, although viruses can change substantially over time in host-specificity and virulence, shrimp virus infections in humans are unlikely to take place. However, factual scientific evidence that would completely rule out this possibility is lacking. Some estuarine invertebrates, such as mussels and oysters, can transmit human viral diseases such as hepatitis and bacterial diseases such as those caused by *Vibrio* spp. Accordingly, another possibility to consider when addressing human health issues, is that virus-infected shrimp

may be less able to control the-proliferation of certain components of their associated bacterial flora, such as *Vibrio* spp., and thus become vectors of microbes that are pathogenic to man.

(8) Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of virus in shrimp and the environmental media?: In the past, tests for the detection of shrimp viruses have yielded mixed results with regards to reliability. Bioassays, histological examination and serological methodologies have been applied alone or in combination but their specificity and sensitivity have been difficult to assess. Substantial progress, however, has recently been made in the development of fast, specific, and sensitive molecular identification and quantification method for the diagnosis of viral diseases in shrimp. Particularly, PCR-based and DNA hybridization technologies have proven extremely useful in this regard (Chang et al 1996; Lo et al, 1996a,b; Loy et al 1996; Wang et al, 1996; Nunan and Lightner, 1997; Hasson et al, 1997)

(C) VIRAL PATHWAYS AND SOURCES

AQUACULTURE

(9) US aquaculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquiculture operations as a source for the virus? It is clear that aquiculture operations suffer from catastrophic outbreaks of viral disease, but unquestionable data on the transmission and establishment of nonindigenous viruses in the environment are not readily available. Therefore, the hypothesis that aquaculture of nonindigenous shrimp constitutes a source for virus spreading to the wild shrimp populations, lacks the necessary factual evidence at present time. A small number of cases of viral and bacterial disease in wild shrimp populations have been proposed to originate in coastal aquiculture or processing operations. In the case of the Mexico's Gulf of California based on the available evidence, it has been proposed that IHHNV transmitted from animals farmed in coastal ponds and hatcheries, may have caused a decline in wild *P. stylirostris* shrimp populations. Interestingly, Mexico does not allow the aquaculture of nonindigenous shrimp species, and in this example this policy may have aided in the transmission of viral disease from the aquaculture setting to the environment if this was the case. Accidental releases to the environment of nonindigenous

shrimp species have been documented in the US aquaculture operations. Furthermorc, under shrimp aquaculture systems in which ponds for high density rearing and waste disposal sites are open to the environment with wastewater routinely discharged directly into coastal waters, it is likely that potentially pathogenic viruses will spread into the environment. Under those conditions, the improbable event of a nonindigenous virus becoming established in the environment may become possible if repeated effluent discharge takes place over time. In this context, it is questionable whether shrimp aquaculture can operate in coastal areas without posing a threat to native shrimp, fish and wildlife stocks in surrounding bay and estuarine ecosystems. However, with proper management practices that include biosecurity and containment measures, continued disease-monitoring and careful treatment of the waste, the risk can be minimized. Finally, there is insufficient scientific knowledge concerning species-specificity of the viruses and the dynamics of their transmission in the environment to make any accurate predictions of the potential hazard of coastal pond shrimp farming.

(10) It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations: usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquiculture and wild shrimp populations, with regard to shrimp viruses? It has been documented that viruses that infect "domesticated" shrimp species such as *P. monodon,* can cross-infcet wild US shrimp species under experimental conditions or in intensive rearing ponds. It is not clear that this can happen in the environment, but the potential for this happening can not be ruled out

SHRIMP PROCESSING

(11) Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus? About 80% of the shrimp processed in the US is imported. Because some foreign aquaculture operations will harvest a pond at the first sign of disease and commercialize the product, the Likelihood of infected shrimp being processed in US seafood factories is relatively high. Some processing operations consist of “unloading/shipping” plants and their potential as virus sources are small. In some others, the shrimp is thawed, peeled, deveined and repackaged. In the latter processing scenario, potentially infectious waste is

produced and, if not adequately treated, may represent a significant source of virus. In some facilities, wastewaters are routed through sewage treatment plants, that inclue chlorination and hydrogen peroxide injection, Before the effluent is discharged in the environment. Untreated solid waste may be used in landfills and, if infected the potential of transmission to aquaculture facilities by scavenger birds cannot be ignored Anecdotal evidence indicates that in the Gulf coast, a *Vibrio* sp. outbreak in wild shrimp was associated with areas where presumable infected shrimp harvested in Texas was processed

OTHER POTENTIAL SOURCES AND PATHWAYS

(12) Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure? It has been proposed that because imports of raw frozen seafood are commercialized independently from proceessing plants, and their waste may eventually reach landfills, dumpsites and waterways, they may represent a potential source of exposure that is not subject to adequte monitoring for virus infection. It should be considered during the decision-making process that if surveillance of the imported products resulted m labeling of the packed seafood as virus-infected a serious consumer perception problem may be established, and the impact on this sector maybe considerable.

(13) What sources other than aquaculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop? A number of additional sources and vectors have been proposed, including infected shrimp as bait in recreational fishing, scavenger bird feces, human feces and ship ballast water, although their relative importance in virus transmission remains to be determined. Possibly, bird feces should be the priority topic for discussion because there is documented evidenece about the presence of virus and it could represent a viral pathway from cultured shrimp to the wild shrimp populations and vice-versa

(14) Is manufactured shrimp feed a potential viral source, or is the processing temperature sufficient to rule this source out? Because most shrimp farms in the US use exclusively pelleted shrimp feed this represents a potential viral source. However, the manufacturing process subjects the feed to temperatures between 170 ^{0}F and 230° F, which are sufficient to destroy most viruses. This should be determined experimentally and the issue resolved timely.

STRESSOR EFFECTS

(15) How should the available evidence concerning the effects of introduced viruses on wiId shrimp populations be interpreted? The factual documented evidence concerning the presume of introduced viruses on wild shrimp populations is certainly not overwhelming, and it remains unclear that any efffects have taken place as a result of these if in fact have occurred. In the GuIf of California a decline in wild *P. stylirostris* populations, has been associated with the detection of IHHNV, but it remains unclear that the virus may have been the cause, and other environmental factors and overfishing may havee compounded the problem. In fact wild shrimp populations in areas in South Carolina and Texas where outbreaks of viral disease such as TSV and IHHNV have taken place in coastal aquaculturc operations, have not shown any signs of decline in following years. However, this evidence does not demonstrate that viral transmission or disease have not occurred in the wild shrimp populations. Therefore, the evidence has to be interpreted with caution and extensive research is needed to determine (a) the presence, virulence and load of "native" and nonindigeaous viruses in wild shrimp populations and (b) the environmental conditions under which these may produce disc.asc m the aforementioned wild populations.

(16) There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in US waters. How should this data gap be evaluated in a risk assessment? Unfortunately, this is one of the critical aspects of a risk assessment and that would require considerable investment of resources and research efforts. Most of the "new" viral diseases recently described, have become patent m aquaculture settings and in many cases with catastrophic consequences. However, it remains unclear if these viruses can be present in the wild shrimp populationsor in other species, with insignificant or unnoticed effects. Therefore, sensitive and specific, quantitative molecular tools should be applied to the assessment of the presence and levels of native and introduced viruses in the wild shrimp populations and other sympatric crustacean species. Similarly, a similar monitoring initiative should be developed in low and high density mating ponds in aquaculturc operations. At present time, however, this data gap should be evaluated with caution, and it should be assumed that the potential for the establishment of pathogenic shrimp viruses in wild shrimp populations in US waters is substantial.

(17) How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future? Fluctuations in coastal wild shrimp populations not exposed to aquaculture operations should be determined and the baseline data compared with those obtained with wild shrimp populations from areas where nonindigenous shrimp farming takes place, and particularly where viral disease outbreaks have occurred. Differences in the population profiles during or after disease outbreaks may provide insight in the effects of introduced viruses in the wild shrimp populations. This has to be accompaniedby careful sampling and monitoring of actual presence of the specific virus in the wild shrimp population in order to make the comparisons meaningful.

(18) How important are potential viral effects on non-shrimp species? It is well documented that some viruses can infect other crustacean species. For example, white spot syndrome baculovirus (WSBV has been detected by PCR techniques in cultured and wild shrimp *[P. monodon, P. japonicus, P. penicillatus* and *Merapenaeus ensis* (sand shrimp)], prawns *(Macrobrachium rosenbergii),* crabs *(Charybdisferiatus, Porturus pelagicus and P. sanguinolentus)*and other arthropods, in different Asian countries (Lo et al, 1996). Therefore, the potential threat of shrimp viruses for non-shrimp species in the US and the ecosystem overall, cannot be ruled out.

COMPREHENSIVE RISK ASSESSMENT AND RESEARCH NEEDS

(19) How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will add significantly to the information presently available? There is no doubt that a comprehensive risk assessment would contribute to a more useful management of the shrimp virus problem. 'Ihe limitations to conduct such type of initiative reside in the quantity and quality of the available data, resources, and particularly, time. Therefore, in the present situation it may be important to focus on a more limited set of goals and assessment points in order to conduct a risk assessment that will permit limited but immediate management decision making.

(20) What type of assessment should be conducted next (e.g.$_q$ quantitative risk estimates using shrimp population models), and what

would he the likely frame and cost? To conduct a quantitative risk assessment as the second step of the process would be logical. However, the scientific tools would have to be developed, applied and a large amount of data collected before this initiative could be carried out in a meaningful manner.

(21) Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios? Yes. But again, this type of risk assessment can only be conducted with data that is only partially available.

(22) Summarize the critical research needs for completing such a risk assessment.

1. Continue the development of sensitive and specific molecular probes for the known viruses that effect crustaceans, particularly nonindigenous and native shrimp species. Develop quantitative diagnostic methodology, such as competitive PCR.

2. Identify markers for stress and acute phase response in shrimp species, such as inducible peptides, protease inhibitors and lectins. Develop the molecular tools to detect and quantitate these markers in cultured and wild shrimp.

3. Apply those molecular tools to determine baseline occurrence and levels of viruses and stress indicators in wild shrimp and other crustacean species. Compare the information with that obtained from cultured shrimp, in healthy ponds and duing viral disease outbreaks.

4. Develop and apply population models that will explain and aid in predicting natural variability of US wild shrimp populations.

5. Continue and expand experimental work on the species-specificity, infective doses and virulence of the viruses of interest, together with viability outside the host and dynamics of disease transmission. Correlate this information with molecular data on stress markers.

6. Expand efforts to gain insight in the inducible recognition and effector factors that mediate shrimp immune mechanisms and their failure to clear/inactivatc their specific pathogens.

7. Apply the molecular qualitative and quantitative tools and bioassays for virus viability to examine possible sources and pathways such as imported processed shrimp, farm pond water, sediments, scavenger bird and human feces. shrimp feeds, ballast water, and others.

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Shiao Wang

1. How well does the management goal reflect the dimensions of the shrimp virus problem?

2. Some have suggested modifying the assessment endpoints to emphasize potential risks of shrimp viruses to non-shrimp organisms and the larger estuarine ecological system or, alternatively, to the aquiculture industry. Please comment on the assessment endpoints as the focal point for the ecological risk assessment.

   There is good evidence now that WSSV infects several non-shrimp arthropods. The effects of WSSV on these non-shrimp hosts are unknown. If the effects of WSSV infection on all arthropods are similar, then there is real cause for concern with regard to estuarine ecosystems. However, our knowledge and fear of shrimp-infecting viruses are based almostly entirely on either laboratory or intensive aquaculture observations. Such observations should not be used to predict what would occur in natural ecosystems.

3. It has been suggested that the scope of the proposed risk assessment is too narrow and that it should be broadened to consider the impacts of such stressors as alternative land uses and seafood production methods in coastal areas. Please comment on this suggestion.

   I think this suggestion warrants consideration. We should learn from problems many foreign countries are currently experiencing with shrimp aquiculture, both ecological and viral, and broaden the risk assessment process to consider potential impacts on coastal areas.

4. How relevant to virus effects on wild populations is information on infectivity and effects that is derived from laboratory or intensive aquiculture operations?

   Information on infectivity is relevant but information on effects is not, in my personal opinion. I can't imagine these shrimp viruses existing in the wild as naked viruses which means that these viruses are a part of the microbial ecosystem. Each virus-bacterial host

complex co-exist with many others, probably sharing and competing for similar resources. Diversity is maintained by trophic interactions and resource limitations, preventing the dominance of any one single species. The effects seen in intensive aquiculture operations, an artificial environment result from the dominance of one particular virus that is infectious to shrimp. Such dominance, in my opinion, would not take place in nature.

5. How likely is it that exposure of wild shrimp populations to viral diseases could lead to the development of immunity and reduced effects on population survival over time?

Anecdotal observations would suggest that this is quite likely. For example, when BP was first reported by Couch in 1974 in pink shrimp (Penaeus duorarum) from Cedar Key, Florida, 20% of feral shrimp were infected. Approximately two years ago, Dr. Kenneth Stuck of the Gulf Coast Research Laboratory collected pink shrimp from the same location and others along the Gulf Coast of Florida looking for BP. Although many hundreds of shrimp were examined, less than 1% were infected with BP. One possible interpretation is that over 20 years, pink shrimp susceptible to BP infection have been selected against and the current population is composed predominantly of those more resistant to infection.

Furthermore, minutes of the 1997 Stakeholder Meetings on the Report of the JSA Shrimp Virus Work Group reported that a severe decline in P. stylirostris population in the Gulf of California was associated with the occurrence of IHHNV in the wild population. The P. stylirostris population has since recovered and returned to normal. Note that an association was reported; no one said that IHHNV was the cause. Nevertheless, IF the decline was due to IHHNV, the recovery would suggest that selection took place and that the present population is more resistant to IHHNV.

6. How can the strong influence of both natural and non-viral anthropogenic factors on shrimp populations be separated from risks associated with viral stressors?

I don't think it is possible. Although laboratory studies have shown that exposure to anthropogenic stressors (such as toxins and pollutants) does not always increase the susceptibility of shrimp to viral infections, it is extremely difficult to convince someone that stressed shrimp are not more susceptible. Therefore, I don't think it will be possible to partition the effects of non-viral anthropogenic factors from viral stressors on shrimp populations.

7. Can human health effects from shrimp viruses be ruled out as a concern? Why or why not?

Yes, the viruses are quite host specific. In addition, the immune system in humans is much more advanced compared to invertebrates and thus should be able to inactivate the viruses.

8. Are the available identification techniques for shrimp viruses reliable enough to allow definitive conclusions to be drawn about the occurrence of viruses in shrimp and environmental media?

Yes, I would say that the identifications techniques (PCR and antibody-based) we have for TSV, IHHNV and WSV are quite accurate in terms of identification. I am still concerned though about making false negative conclusions that are based on PCR results. Shrimp tissues contain unidentified compounds that inhibit DNA polymerase. These compounds can be difficult to separate from DNA thus a negative PCR reaction does not automatically rule out the presence of the virus. Including an internal positive control helps but the problem is still a concern. I haven't kept up about the diagnosis of YHV.

9. U.S. aquiculture operations have had problems with viral diseases for several years. How does information from local wild shrimp populations support or refute the importance of aquiculture operations as a source for the virus?

I don't think we have enough information and experience to make that determination. In terms of scale, we have not had the type of problems that Asian countries are experiencing. There is little doubt that aquiculture operations provide a more concentrated source of pathogens because of their dense or intense nature. However, once discharged into the natural environment, the effect of dilution and microbial interactions on viral infectivity is unknown.

10. It has been widely held that it is highly unusual for domesticated animals to infect wild animal populations; usually it is the other way around. How well does this observation apply to the relationship between shrimp in aquiculture and wild shrimp populations, with regard to shrimp viruses?

I don't think this observation would apply in the case of shrimp. Pathogens dispersed via water are much more difficult to contain than those on land. Farmed animals such as cows and chicken are monitored much more closely thus pathogens have little chance to spread on the farm, much less to wild populations. This is completely different from the way shrimp is cultured.

11. Some believe it likely that shrimp processing operations have processed virus-infected shrimp from foreign sources for several years. How does information from local wild shrimp populations support or refute the importance of shrimp processing as a potential source for the virus?

I don't know the importance of shrimp processing as a potential source for the virus. I don't doubt that shrimp processing operations have processed virus-infected shrimp from foreign sources. However, there is not enough known about viral persistence in the natural environment to determine whether shrimp processing is a significant source of viruses. Studies on the dynamics of virus abundance in coastal seawater have shown large temporal fluctuations in matters of 10-20 minutes. Processing operations can introduce virus to the

environment, whether the virus persists long enough to infect natural populations is not known. My feeling is that shrimp aquiculture operations present a more significant source of virus in terms of abundance while processing operations present a more significant source in terms of introducing new viruses from afar.

12. Should the retailers who distribute (rather than process) shrimp products receive additional evaluation as potential sources of exposure?

    No, unless we're talking about bait shrimp.

13. After considering the sources addressed in the shrimp virus report, what sources other than aquiculture and shrimp processing are most critical for evaluation in a risk assessment of shrimp viruses? Given time constraints, which of these should be the focus of discussion at the workshop?

14. Is manufactured shrimp feed a potential virus source, or is the processing temperature sufficient to rule this source out?

    I have no personal experience with feed manufacturing but this source should be ruled out. Not only will high temperature inactivate viruses but, at least in the case of BP, the simple process of dry will also do the same.

15. How should the available evidence concerning the effects of introduced viruses on wild shrimp populations be interpreted? (For example, what was the role of IHHNV in the decline of shrimp populations in the 1980's in the Gulf of California? What about TSV release from aquiculture into the wild in South America?)

    These are associations where no cause and effect can be shown. In my personal opinion, we should not extend our observations on the effects of viruses on shrimp in the laboratory or in

aquiculture operations to what might take place in the natural environment. The effect of viruses on shrimp populations in the natural environment lies at the heart of the risk assessment process and more research is needed.

16. There is presently a lack of basic data on background levels of pathogenic shrimp viruses in wild shrimp populations in U.S. waters. How should this data gap be evaluated in a risk assessment?

I would disagree with the statement. Information concerning BP, a naturally occurring baculovirus in U.S. waters, is currently available. The natural infection cycle (when infected shrimp occur along the coast each year and the size distribution of those infected) have been well characterized by Drs. Overstreet, Lotz and Stuck at the Gulf Coast Research Laboratory. However, I do think there is an important data gap that needs attention. Although the occurrence of BP has been well characterized, its effect on wild shrimp population dynamics is unknown. Infected shrimp that die or become more susceptible to predation are quickly eliminated and thus never accounted for. Whether this is important in terms of overall shrimp population dynamics needs research.

17. How can changes in wild shrimp populations be used to interpret the effect (or lack of effect) of introduced shrimp viruses? How could shrimp population models be used in the future?

See responses to questions 15 and 16.

18. How important are potential viral effects on non-shrimp species?

See response to question 2.

19. How will a comprehensive risk assessment contribute to management of the shrimp virus problem, i.e., will it add significantly to the information presently available?

Risk assessment is out of my area of expertise. I'll go directly to question 22.

20. What type of assessment should be conducted next (e.g., quantitative risk estimates using shrimp populations models), and what would be the likely time frame and cost?

21. Should a future risk assessment consider the risk reduction potential of a range of treatment options associated with specific exposure scenarios?

22. Summarize the critical research needs for completing such a risk assessment.

I think there are two critical questions that we need to address. First, what is the effect of virus infections on the population dynamics of wild shrimp? Second, what is the natural history of viruses that are pathogenic to. shrimp in the natural environment? Although empirical evidence to answer the first question will be difficult to obtain, some information is available to model virus-shrimp dynamics in the natural environment. At least for certain viruses, there is information concerning the following areas: 1) effects of shrimp age and condition on susceptibility to infection; 2) viral persistence in shrimp; 3) sublethal effects of viral infections; 4) the effects of genetics on viral resistance. Such information, along with fisheries statistics on the influence of predation and environmental conditions, should be useful in models to determine whether viruses play a significant role in wild shrimp population dynamics.

The second question may be of greater importance in risk assessment. We can not assess the risk of viruses introduced either by shrimp processing operations or by aquiculture if we do not understand what happens to viruses that are released into the natural environment. With the availability of current molecular techniques to identify and to quantify viruses, a definitive answer to this question can be obtained. The importance of this question with regard to risk assessment warrants additional research.

The premeeting comments of Max Summers that follow were not received by ERG in time to be included in the assembled premeeting comments distributed to peer review experts prior to the workshop.

Preliminary response to "Charge to Panel Members"

A. Diagnostic techniques for shrimp pathogens

1) How good are the diagnostic techniques relative to specificity and sensitivity?
2) Can these detection and identification tests be equally applied for all pathogens of concern?
3) With the diagnostic tests available, what is the level of detection and identification suitable for reliable risk assessment (endpoint) analyses?

B. With highly sensitive, specific and reproducible diagnostic techniques, one can more quantitatively and qualitatively develop feasible risk assessment data for:

A. Management goals, conceptual risk models:

2. Potential to spread to U.S. shrimp populations.
3. Potential to spread to "non-host" populations.

B. Viral stressors and factors regulating shrimp populations:

4. Correlation of empirical laboratory data with virus infection and spread in wild populations.
5. The development of disease resistance.
6. The effects of natural and non-viral anthropogenic influences for virus introduction and spread.
7. Potential effects on human health.
8. The credibility and reliability of shrimp virus diagnostics - this area needs a constructively critical and comprehensive assessment by epidemiologists/epizoologists who are expert in these applications for monitoring experimental and natural populations of animals and man. I would suggest a team of individuals working with shrimp pathogens and those who are expert and knowledgeable of predicting potential for pathogen introduction and spread in human populations.

C. Viral pathways and sources:

10. Diagnostic tools are key to evaluating the potential for virus spread in exposed shrimp populations.

D. Comprehensive risk assessment and research needs:

22. A critical assessment of diagnostic techniques; and the program of how such is to be used and implemented to detect/identify the target pathogen in any potential source of introduction and spread within populations.